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PROGRESS IN NUCLEAR ENERGY

SERIES VI

BIOLOGICAL SCIENCES

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PROGRESS IN NUCLEAR ENERGY

SERIES VI

BIOLOGICAL SCIENCES

VOLUME 1

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NOTE

In reference lists and elsewhere "Geneva Conference Paper" refers to papers presented at the International Conference for the Peaceful Uses of Atomic Energy, held at Geneva, in August, 1955.

FOREWORD

By JOHN C. BUGHER, M.D.

The Rockefeller Foundation, New York

NUCLEAR energy, that genie of modern science, has many faces. Conceived in the minds of philosophers, mathematicians and experimental physicists, born of strife, it now, in its youth, begins to show its potential for profoundly affecting the lives of men and the structure of peaceful societies. The origins of this field of science lie in many lands and in many disciplines. No single group of scientists in any country has been exclusively responsible for any one of the major developments. The origins are international. Science itself observes no national boundaries. It is both natural, and to a certain extent inevitable, that these matters should be of concern and interest to all peoples of the earth.

The International Conference on the Peaceful Uses of Atomic Energy at Geneva was called in recognition of these fundamental considerations. Many different lines of approach had to be incorporated. Some of them led to the consideration of matters many years, perhaps decades, in the future. Others dealt with the impact of profound industrial change on the technology and productivity of segments of society. With these, however, there had to be considered those ways in which this new resource comes to affect the lives and relationships of people as individuals. These are the applications in medicine, in biology generally, and in the improvement of those technologies by which men live. Many of these applications are already in widespread use; others are in the pilot stage of their development; while still others are only dimly foreshadowed.

Just as in the concepts of the nature of physical substance, there has been wrought a profound change in the understanding of life. Much of this change is attributable not only to the new tools which nuclear science has made available to the biologist, but also to the modification of the patterns of thought which the results of scientific investigation have compelled. Today the phenomena of life are analysed in terms far more dynamic than were those of a few years back. This change, spectacular though it is, has occurred quietly but rapidly in nearly all phases of biological science throughout the world. One of the more impressive aspects of the Geneva Conference is to be found in the depth of understanding among scientists from so many geographical areas and speaking in such diverse languages.

The immense amount of factual and theoretical material presented at this conference is beyond the available time of most scientists to assimilate. It has appeared most important that the significant matters of the sessions at Geneva be brought together in as compact a form as possible, so that the material may have the widest availability. In these volumes the reader will find distilled for him the essence of the discussions. For the scientific detail with which the specialized scholar will wish to acquaint himself, the references to the individual papers will bring to his hand the entire resources of scientists throughout the world.

In these chapters, there is combined the practical with the theoretical; the present uses with the future possibilities. The problems of the operation of nuclear facilities, of personnel protection and of environmental radioactive contamination have been considered in detail. There has been a clear recognition that this new industry brings to the foreground the questions of environmental effects upon whole populations. These questions have always existed in relation to industry, but they have been dramatically highlighted by the great increase in magnitude of the amounts of toxic substances involved.

Matters considered here touch on every phase of the relation of man to his environment. The productivity of the land and of the sea, the conversion of solar to chemical energy by way of enzyme systems, the pace of evolution, are all areas of investigation that are opened by the radioactive tools. All the natural sciences become inextricably interwoven. The agriculturist turns to the soil scientist on the one hand and to the geneticist on the other. Meteorology and oceanography contribute to the solution of terrestrial problems, and all find that the tempo of their sciences has been enormously increased by the resources of the nuclear physicist and engineer.

As a result of the efforts of the authors of the various chapters to compress into small volume the maximum amount of information, the reader should the more easily appreciate the interplay of one discipline upon another. One of the greatest results of this conference in the biological field must surely be the vivid demonstration of the interdependence of the areas of knowledge.

FOREWORD

By V. R. Khanolkar

Director, Indian Cancer Research Centre

As a result of the phenomenal growth of our knowledge of nuclear physics, during the last two decades, a new and powerful tool has become available to research workers in Biology. It is unfortunate that some of this knowledge has important bearings on the production of weapons of war and had therefore to be shrouded in secrecy.

The United Nations Conference at Geneva in August 1955 marks a turning-point towards a well-concerted pooling and exchange of information on atomic energy, in its relation to a wide area of knowledge, extending over many fields of human endeavour.

The production of artificial radioactivity has placed at the disposal of man a wide variety of radioactive elements which could be employed for a better understanding of many processes in living organism. A planned use of radioactive substances is solving several perplexing problems of basic importance and may probably be instrumental in alleviating some of the ills that flesh is heir to. On the other hand, it is creating newer and hitherto unknown problems which are of concern to the human race. It is becoming increasingly clear that man and his domestic animals are most susceptible to the harmful effects of ionizing radiation. It is regrettable that our knowledge of most of these effects on man are still very inadequately understood. A long latent period, between the exposure to radiations and a clinical manifestation of its consequences, which may be as long as twenty-five years, has held up our progress for some time.

The effects of high intensities of radiation, intentionally inflicted or suffered through ignorance, are being carefully studied in many countries. The best guide for protection from such effects is the past experience of damage done to man and animals and it may be hoped that in the future, the deleterious consequences of over-exposure to radiation will be encountered only as a result of unavoidable accidents or those following upon radiotherapy. The installation of power reactors in many countries would undoubtedly increase the radioactivity of the waters, the soil, the atmosphere, and the living beings inhabiting them.

The use of radioactive isotopes in a study of the metabolic processes in plants such as respiration, photosynthesis, biosynthesis of plant pigments and translocations, particularly with regard to root absorption and nutrition has recently yielded valuable information. The disintegration of organic fertilisers and their uptake from the soil by plants has led to the discovery of an interchange of physiologically active substances between soil microorganisms and the roots of plants. The practical applications of this knowledge towards an improvement of soils and crops holds promise for the future, in increasing the food supply on earth for keeping pace with an increase in world population. The aim of the plant breeder is different from that of the human geneticist. The plant breeder is not perturbed by wiping out " by a sort of mass killing, 99 per cent or more of the individuals in an experimental field" to save and select the remaining mutants which may possess beneficial characters. It is likely that the next few years will witness in an ever-increasing measure, an elaboration of methods for inducing specific mutations, in plants of economic importance.

The carcinogenic effects of ionizing radiations of low intensity on large human and animal populations are relatively unknown and therefore still unpredictable. Exact information concerning the rate of mutation induced by such radiation in mammals is still very meagre. A good deal of experimental work is being carried out on certain viruses, some of the lower fungi, fruitflies, and recently on mice. These studies have considerable merit and theoretical significance. It may, however, be extremely hazardous to transfer conclusions drawn from one species of living beings to another. However, if similar results accrue from different species of animals, an extrapolation of results may rest on sounder basis. Careful and long-term surveys of diseases and abnormalities resulting from occupational or therapeutic exposure to radiation are necessary in man. It is now necessary to organize a research programme with three main objectives: (1) basic studies on mutation at different levels of plant and animal evolution, (2) studies on animal populations, and (3) studies on human populations exposed to low intensity but long-continued radiation. These problems could be tackled either by making continued observations on individuals or by a study of differences between population groups which during the normal course of their life have been exposed to low levels of irradiation, over long periods of time. Differences in the normal or background radiations arise either from variation in intensity of cosmic radiation, depending on the altitude of any particular locality or from the presence of radioactivity in rocks such as uranium in granite or thorium in monazite sands. It is likely that a study of plant and animal life inhabiting such areas and a comparison with the fauna and flora of control areas under similar environment but without corresponding radioactivity may yield very valuable information. Such a project of study would demand international cooperation and an active collaboration between scientists in different parts of the world, if any worthwhile information is to be collected. It is gratifying to note that the United Nations are actively interesting themselves in such studies and a panel of scientific experts is at present engaged in laying down the basis for such a programme on a world-wide scale.

BIOLOGICAL SCIENCES

1

MODES OF RADIATION INJURY; BONE AND CARCINOGENESIS; AND STERILITY AND FOETAL DAMAGE

By Austin M. Brues

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Abstract—1. *Modes of Radiation Injury*: Many of the manifestations of the acute radiation syndrome are attributable to injury of the self-replenishing tissues. The haematopoietic system is especially important. Delayed effects range from the shortening of the life span to the development of specific changes, notably neoplasms. Chromosomal injury to developing germ cells may give rise to genetic alterations in succeeding generations.

2. *Carcinogenesis and Metabolism of Bone-seeking Isotopes*: Tumour induction depends on the deposition of the radioactive material in a suitable tissue. Neoplasms may result from direct injury by radiation or indirectly through hormonal or other mechanisms. Bone tumours are associated with radioactive substances that have a metabolic similarity to calcium. Radium, mesothorium, and radiostrontium are especially important in man.

3. *Effects on Reproductive System and Foetus*: X-irradiation during uterine life may give rise to a high frequency of developmental abnormality. Spermatogenesis was disturbed in dogs given ten times the industrial maximum permissible limits for over three years.

1. MODES OF RADIATION INJURY

The many papers proffered in this session of the Geneva Conference on the Peaceful Uses of Atomic Energy, including those not read at the Conference, serve to give a fairly clear and comprehensive picture of the nature of radiation damage to man and to the other higher animals. Information of this sort is important in setting permissible dosages and in the search for agents useful in the therapy of those who become overexposed to radiation through the inevitable, though occasional, accidents which are bound to occur in an expanding nuclear industry. In addition, it is gratifying to note that studies of this sort have, in the several countries presenting material, been carried out largely on the level of basic physiology, thus making significant contributions in basic science.

The first paper, presented by the writer of this review(1), embodies a rather general discussion of the various ways in which radiation injury manifests itself, and of the basic underlying lesions. It is pointed out that many of the manifestations of the acute fatal radiation syndrome are attributable to the destruction of, or inhibition of function of, the self-replenishing tissues, which may in general be identified with those that have been called "radiosensitive".

The predominant role in the acute radiation syndrome has been assigned, ordinarily, to the bone marrow. From the point of view of acute radiation death (occurring a week or more after a sufficient dose of total body irradiation) the agranulocytosis and decrease in blood platelet concentration are significant from the standpoint of failure to maintain a sterile internal environment, and because of the bleeding tendency which may prove fatal for various reasons. Additional evidence is based on the responses of the blood granulocytes in species of widely varying radiosensitivities, the relation of these cells to the prophylactic value of estrogens, and the results of therapeutic measures (injection of material from bone marrow and spleen) which promote recovery of the marrow.

The lymphocyte is another sensitive cell; but its relation to acute radiation death is less well understood, since its functions in the normal economy of the body are still debatable. The gastrointestinal tract, another tissue in which self-replenishment is important, will be treated in discussions of other papers.

Other tissues in which the requirement of self-replenishment is important, include the hair, which may be lost temporarily through cessation of growth, and the gonads, where (in the male at least) temporary sterility is a consequence of irradiation in the median lethal range. Damage to growing tissues is probably not on a basis of inhibition of cell division so much as it is due to injury to the chromosomes, which is visible in a majority of cells treated in the range which is lethal to the higher animals.

Cold-blooded animals show a sequence of radiation effects similar to those manifested in warm-blooded species, but the rate of their development is retarded at reduced temperatures.

The initial radiation response represents a mode or modes of injury different from the above and, except after very high radiation doses, or in certain species, is not fatal. It involves both effects on the autonomic system and biochemical alterations.

Subacute and chronic effects are also qualitatively different. Larger total doses may be accumulated due to what is called a recovery factor. While the haematopoietic system may support itself for a long time through replenishment, it may eventually lose this capacity. Leukaemia, particularly noteworthy in the mouse, appears related to haematopoietic injury in general, rather than to damage to specific cells; this is shown by the fact that protective measures to the blood-forming system reduce the incidence of leukaemia out of all proportion to the amount of tissue protected.

The most delayed effects are noted either by shortening of the average life-span of a group of animals, or by the development of specific changes, notably tumours, in increased numbers. More clear-cut evidence of such late changes and of their etiology is seen following local irradiation, particularly when this is achieved by the use of absorbed isotopes.

The various modes of injury, as discussed by the writer, will receive special emphasis in relation to forms of protection and therapy. For example, those agents and procedures which protect specifically the haematopoietic organs act against acute haematologic death and against leukaemia, but are probably ineffective against other delayed changes.

Sir ERNEST ROCK CARLING(2) discussed the modes of radiation injury from the standpoint of the physician charged with practical responsibilities for prevention, diagnosis, and treatment of radiation injury. While the effects of the various forms of radiation on cells are qualitatively similar, recommendations for the prevention of injury by these forms must take into account a great variety of data, in the case of external radiations, penetrating power, density of ionization, parts of the body affected, and the time relations of the exposure; for radiation by absorbed isotopes, a detailed knowledge of their metabolism by the body, and other related physiological information. In the early days of X-ray therapy, serious results occurred through failure to appreciate the importance of the volume of tissue irradiated. The radon breathed by uranium miners was eventually discovered to be carcinogenic, as well as intake of radium and thorium.

While there is a wealth of experimental material bearing on the question of radiation sickness and injury on all levels from the total body to cellular molecules (particularly the chromosomes) and much information has been obtained which is approximately applicable to man, the natural variance in sensitivity (exemplified by the use of the LD_{50}) indicates that some will survive, and the basis of this needs to be better understood.

The question of the true significance of genetic mutations to the human race was raised, and it was suggested that all mutations may not be of a deleterious nature (ROCK CARLING's intimation that geniuses as well as mental defectives might result from mutations was disputed by geneticists discussing the paper; one of these discussants took the opposite extreme, that the genius may be an individual harbouring a minimum of mutations).*

The diagnosis of radiation injury is a difficult one and past radiotherapeutic records have not been helpful owing to efforts to avoid damage and to the delimited radiation fields used in therapy. Most of the usual laboratory procedures yield little information as to the course of radiation damage, with the exception of examination of the blood cells; in continuous radiation this, too, leaves a great deal to be desired, since a well-maintained blood count may suddenly be followed by an intractable anaemia. The level of exposure must therefore be held below the maximum permissible.

Because of the inevitable increase in radiation exposure in the future, protective codes are of great importance. These will necessarily undergo change as we learn more about the subject; for example, past recommendations as to frequency of blood counts have been thrown into doubt. Such regulations will have to take into account the many other environmental hazards generally accepted as reasonable. It has been proposed that personal records of radiation exposure throughout life may become advisable. Among important questions not yet resolved are: whether an additional factor of safety needs to be introduced where large-population exposure is under consideration, and at what value the dose-rate assumes importance.

Both this and the previous speaker gave emphasis to the high general level of safety and the rarity of accidents in nuclear energy establishments, due to the foresight and determination of the many national groups responsible for safety in this field.

The third paper of the session, by I. A. PIGALYEV(3), discussed in detail one very important aspect of radiation injury, namely resistance to the infections that accompany it. The widely observed bacteraemia and spread of infections testify to a breakdown in the mechanisms of resistance. Even the bactericidal property of the skin is reduced following irradiation, and it is of note that this declines in a zonal pattern, occurring first over the skin of the abdomen, perhaps related to the early sensitivity of the abdominal organs. In the case of buccal mucous membranes, increased invasiveness has been thought to be related to a fall in the lysozyme content of saliva.

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* The reviewer would like to suggest that both these points of view are drastically over-simplified.

The course of experimental infection is dependent on radiation dose and on the time of the inoculum after irradiation. Bacteraemia (*E. coli*) develops after a large dose (1 100 r to the rabbit) and is stable until death, but only if the infection takes place 2 days or more after irradiation. At lower dosages bacteraemia may be only transient. Infection by non-pathogenic organisms through the tracheal route also results in bacteraemia three to four days after irradiation, a picture similar to that manifested by pathogenic pneumococci in unirradiated animals. Other examples were given indicating the exacerbated course of various experimental infections.

The question of alterations in bacterial virulence as a result of growth in an irradiated host was raised. While past investigators have not observed this, it is thought that the question has not yet been finally settled.

Studies on the effects of internal radiation (polonium or radiothorium) combined with administration of bacterial toxins (tetanus and diphtheria) indicate that there may be a reciprocal influence of the two types of agent, so that mortality is intensified but the manifest effects of the toxin are reduced or eliminated.

Many experiments have shown that a fully formed acquired immunity is unaffected by radiation, the development of immunization is greatly altered. Passively acquired immunity is also reduced. Utilizing both external and internal radiation sources, it has been observed in a variety of experiments that the amount of antibody formed is much reduced and that re-immunization at a later time is relatively or absolutely ineffective. When irradiation is performed a few days after immunization, the antibody titre may rise transiently above control values, but then declines sharply.

That a change in the antibody may play a role is indicated by experiments in which cell nuclei of normal and of irradiated rats were used in sensitization and desensitization of guinea pigs. It was found that nuclei of irradiated donors were relatively ineffective in desensitizing animals previously sensitized by normal preparations, while normal nuclei desensitized fully animals sensitized by either type. Similar experiments in which cytoplasm was used as the antigens, also suggested that irradiated preparations were deficient in certain properties. Irradiation also increases sensitivity to histamine and adrenalin, and the Schwartzmann reaction fails to develop at the height of radiation sickness. Reactivity of various tissues to cholinergic and adrenergic materials is altered by radiation, and these changes may be phasic ones. These effects have been in some instances related to alterations in enzyme activities in nerve endings and elsewhere. These data, together with the observation that radiation reduces phagocytic activity, all indicate the complexity of the immunologic effects of radiation, which appear to reflect reactions of the whole organism.

In addition to the papers presented orally, the subject of the modes of radiation injury was further greatly amplified by a number of other submitted communications; and in fairness to this large and important subject the reviewer has undertaken to discuss them all briefly.

CLARK, JORDAN, & VOGEL(4) presented a series of comparative studies on the effects of fast fission neutrons and cobalt-60 gamma rays on mice. Based on acute mortality statistics (30 day LD_{50}) and in terms of roentgen equivalents, the neutrons are more effective in the ratio of 4·43:1. Splenic weight reduction yields a ratio of 4·42, which may be considered identical. When the two modes of radiation are given in the form of mixtures, the LD_{50} is increased as the gamma component is decreased; this departure from additivity is limited to the first few weeks after irradiation. Neutron irradiation shows a peak in mortality at 5 to 6 days and gamma irradiation at two weeks within the range tested (LD_{20} to LD_{80}). When the treatment time is protracted to 24 hours, mortality from neutrons is unaffected but a larger gamma ray dose is required to kill, resulting in a ratio of biological effectiveness of 6·3. In both forms of radiation, bacterial invasion is seen and is correlated with the respective times of death; streptomycin therapy reduces neutron mortality in the first ten days but not thereafter; while cysteine administered before radiation is equally effective in both cases. It is concluded that the mode of injury differs with the two agents.

BRACE & ANDREWS(5) presented data involving X-ray doses to guinea pigs well above the LD_{50}, and found neurological changes predominant. There appears to be a sharp break between 5 000 r, which leads to progressive depression and death in 5 days, and 6 000 r, which results in hypersensitivity and death in the first two days accompanied by clonic convulsions. Bradycardia and minimal electro-encephalographic changes occurred at doses of 2 500 r. Blood chemical changes were not striking at any of the levels employed; granulocytosis and lymphopenia occurred at all. Shielding experiments showed that the excited state required irradiation of the hind-brain and brain stem; 25 000 r to the fore-brain alone resulted only in the delayed mode. Pycnosis of the granule cells of the cerebellum seemed closely related to early death.

Because of the sharp distinction between the two modes of death, experiments were done using a variety of anticonvulsants and central depressants. When pentobarbital was given in a quantity producing surgical anaesthesia at the time of irradiation, the hyperexcitable stage was lost and survival was prolonged even at 15 000 r, and pycnosis of the granule cells was suppressed.

QUASTLER(6) took up several modes of death, pointing out that each one is masked by another requiring a larger dose but resulting in earlier death; they can be unmasked by local shielding or therapeutic procedures directed to a particular mode. The modes are outlined as follows (in each case followed by the threshold dose and time of death in the mouse): marrow death, 600 r, 12 days; intestinal death, 1 100 r, 3½–4 days; oral death, 1 100 r, 10 days; brain death, 2 000 r, 3 days; 12 000 r (probably another mode; cf. ref. 5), less than 3 days.

The mechanism of the intestinal mode was discussed in detail. It is seen over a wide dosage range above 1 100 r, and up to 6 000 r the time of death remains the same. If the head is shielded, intestinal death is the predominant mode up to 30 000 r. It is also determined by irradiation of the intestine only, or even that of a part of the small intestine. It is suggested that it results from the cessation of mitosis in the crypts, which is necessary to keep the villi intact through replacement of cells; the end-result is due to a combination of factors, e.g. loss of fluid and escape of proteolytic enzymes. Various feedback mechanisms which minimize the denudation of the epithelium were mentioned.

GAUDE & COURSAGET(7) discussed experimental studies on animals irradiated by cobalt-60 gamma rays, bearing on immune processes. No differences were found in the phagocytic behaviour of leucocytes obtained from animals irradiated with various dosages up to 250 r, at intervals of time from 2 hours to 2 weeks after irradiation. Formation of antibodies by rabbits inoculated with an antipneumococcal vaccine was found to be depressed as a result of irradiation 6 hours or more before vaccination, where the dose was 400 r or higher. The data suggested increased antibody formation after 100 r.

The LD_{50} for the guinea pigs was estimated as 144 r, but with remarkable individual variation; some died after 50 r, and there was one survivor after 250 r.

FRANK(8) reviewed a large amount of work on the early reactions of dogs, rabbits, and rats to both general and local irradiation. The rapid drop in blood pressure (rabbit, 1 250 r X-ray) was found to be accompanied by a decrease in circulating blood volume and an increase in blood viscosity. Haematocrit values were not reported, but studies of the red cells showed an initial increase in size followed, some days later, by marked anisocytosis—the latter attributed to impairment of blood formation. In vitro haemolysis of red cells by ultraviolet light was found to be accelerated 3 hours after X-irradiation of the whole rabbit. Since it was noted that the pulse-wave velocity, which was decreased immediately after irradiation, became greater than normal during recovery, a study was made of the elasticity modulus of the aorta; this was found to increase and to reach a maximum at 8 to 10 days.

Cerebral oxygen consumption was studied in vivo (rabbit, 1 100 r) by measuring the curve of oxygen content after brief O_2 inhalation, and was found to be markedly depressed. This hypoxia was thought to be responsible for subsequent diffuse gliosis which is observed pathologically. Measurements of impedance and capacitance of the brain during irradiation (rabbit 5 r/minute) showed marked alterations within the first 10 minutes. After 20 minutes impedance fell to a minimum and capacitance rose to a maximum; after 3 hours the situation reversed and was then irreversible after cessation of irradiation.

Partial body irradiation of rats (total body $LD_{100} = 800$ r) showed LD_{100} values as follows: head 2 000 r, lower abdomen 3 000 r, upper abdomen 5 000 r (but cf. ref. 6), chest 10 000 r. Exposure of the head resulted in leucocytosis, and the haemodynamic changes were comparable to those seen in total body irradiation. Cerebral oxygen consumption was depressed. Abdominal irradiation failed to show this, but resulted in changes in cerebral impedance somewhat similar to those following irradiation of the head. Irradiation of the skin of the abdomen with 20 kV X-rays depressed the blood pressure, and this reaction was abolished by novocain infiltration of the area to be irradiated. It was noted, finally, that injection of the head of the rabbit (3 000 r) reproduced the changes in plasma protein distribution that follow that of the total body. It was concluded that further detailed studies of partial-body irradiation effects are desirable in elucidating the mechanism of the radiation syndrome.

STANNARD, BLAIR, & BAXTER(9) reported studies of the irreversibility of damage by alpha emitters, employing polonium and radon (since most other alpha emitters are rather specific for the skeleton, interpretation is more difficult), using shortening of the life-span as a criterion of late injury. It was found that, while about 95% of X-ray injury is reversible if the dose rate is

100 r or less, only about 20% is reversible using polonium in single or monthly injections. These inferences were further borne out by comparison of the data with published data on strontium-89.

FRIEDELL & SALERNO(10) have approached the general question through a study of the toxicity of various isotopes, alone and in combination. It was found that combinations of bone-seekers (P^{32} and Sr^{89}) or of colloids irradiating liver and spleen (Au^{198} and P^{32}—chromic phosphate) are simply additive, while a combination of one of each type of isotope is markedly synergistic in lethal action. It was observed, moreover, that splenectomy has no effect on Au^{198} toxicity but markedly enhances that of Sr^{89}. Splenectomy has an equivocal effect on survival of rats after X-ray at the highest doses, and appears to be very slightly deleterious in the case of the irradiated mouse. The data confirm observations of others that the spleen is more important in recovery of the mouse than of the rat.

BLAIR(11) presented a generalized mathematical treatment of delayed radiation injury (shortening of life span) and applied it to available data from his group and the work of others. The treatment is based on assumptions that total injury is proportional to dose and includes both reparable and irreparable components, that recovery from reparable injury is proportional to its magnitude, and that death occurs when the total injury is proportional to the remaining life expectancy. Using these postulates, it is estimated that irreparable injury from fast neutrons is about 40% of the total, that from internally administered alpha and (probably) beta rays is about 80%, and that from *x* or gamma rays is 5% for late injury and somewhat higher where the dose rate is higher (suggesting, perhaps, more than one recovery function). The half-time of recovery from an initial dose varies with species and may be inversely related to the acute LD_{50}. It is believed that a theoretical treatment of this sort will be valuable in the extrapolation of experimental data to man.

GOWEN presented two papers(12, 13) dealing with the influence of genetic differences on disease resistance and irradiation responses. It appears that susceptibility to mouse typhoid and longevity are unrelated, while there is some correlation between susceptibility to typhoid and to X-ray. Leucocyte numbers are correlated with typhoid resistance, and this relation holds even when the animals are irradiated.

A study of mutants produced by irradiating *S. typhimurium* showed that strains of high virulence tended to mutate to forms of lower virulence and *vice versa*. The results revealed the complexity of factors required for pathogenicity of the bacteria or for immunity production.

Comparison of two strains of mice given radiation by means of nuclear irradiation (nuclear detonations under field conditions) and 98 kV X-rays showed a ratio of about 3 in favour of the former. The longer-lived mice (Ba) were in both cases more sensitive to radiation than the shorter-lived (S strain).

ZUPPINGER(14) discussed mammalian radiation injury on the basis of the early chemical changes responsible for injury. 30 MeV betatron radiations is less effective than 250 kV X-ray for several biological effects. It was noted that injections of haemin into mice after irradiation results in a remarkable increase or acceleration of splenic hypertrophy (measured at 7 days after irradiation) while it results in only a very modest, or perhaps insignificant, improvement in survival.

TSUZUKI(15) described the effects of radioactive fallout on 23 Japanese fishermen. The effects were due to (1) beta irradiation of skin and hair due to externally deposited ash; (2) gamma radiation to the body due to the fallout material; (3) internally deposited absorbed isotopes, particularly radiostrontium. The latter was probably relatively insignificant. External gamma ray exposure was estimated to be between 200 and 400 r. One patient died with liver disorder, and it is not clear whether this was due to homologous serum hepatitis as a result of multiple transfusions or to radiation effects (direct or indirect) on the liver; the other 22 are improving.

2. CARCINOGENESIS AND METABOLISM OF BONE-SEEKING ISOTOPES

NEUMAN(16) discussed the basic knowledge in skeletal physiology gained under the impetus of new research in the fixation of isotopes in bone. The rapid uptake of radiocalcium from the blood, almost complete in an hour, has forced a revision of the older concept that materials are deposited in bone only where it is growing; it is now seen that many ions are passively exchanged for calcium, phosphate, or hydroxyl at the surface of the bone crystals. The surface of these crystals is over 100 square metres per gram, and many of the ions are at the surface and available for exchange. Bone also contains water of hydration, polysaccharides and collagen, which is aligned with crystals.

Certain elements, which tend to form colloidal hydroxides at physiological pH (including the rare earths and actinide elements), concentrate in the organic matrix of growing bone. Those which deposit mainly in the mineral phase (alkaline earths including radium, and some others) do so through diffusion, exchange, recrystallization, and new crystal formulation. Remodelling of the adult bone by the formation of new haversian systems is a mode of deposition which leaves the deposited material quite inaccessible to methods of mobilization and removal. All of these processes are slower in the adult than in the young animal, hence deposition decreases with age. Experiments employing radiosodium indicate that the "available" skeleton decreases from $\frac{3}{4}$ to $\frac{1}{4}$ in the course of body growth.

HOECKER(17) amplified and confirmed the above, with additional observations in the same field. The importance of the spotty deposition of the alkaline earths in the haversian systems is emphasized by the fact that they occur in the human cortical bone but are not seen in the rat, an animal lacking these systems: deposition in the spongy part of bone is, however, the same in both species. Simultaneous injection of radium and alizarin red confirms the belief that radium deposition in cortical bone occurs where bone is being laid down.

Placental transfer of the various alkaline earths shows that they are handled differently in the blood stream: calcium is transferred readily, while with increasing atomic mass the transfer decreases and is zero in the case of radium. Lactation seems to resorb bone, but radium and analogous radioelements after a period of time are only slightly mobilized during lactation; in fact, radiation damage to bone phosphatase may diminish the tendency to resorption. This writer agrees, with the previous one, that methods of removal of radioelements from bone are likely to be very effective only if applied soon after introduction of the radioelements.

CLARK(18) presented a clinical study based on a survey of young patients coming to the clinic with carcinoma of the thyroid. All of such patients in childhood or adolescence coming to the clinic had a history of radiation to the chest, neck, or throat during the first few months or years of life (to a maximum of 6 years).

Similar studies have been made of persons who received X-radiation to the thymus in infancy, and reveal an abnormal incidence of thyroid cancer in late childhood and adolescence. The minimum dosages thus inducing cancer of the thyroid are about 200 r. It appears that the thyroid in infancy is much more radiosensitive (with cancer production as a criterion) than that of the adult.

A review of tumour induction by internal emitters was submitted by FINKEL(19). These tumours are generally local, either in the tissues in which the radioactive material is deposited, or in adjacent tissues absorbing the radiation. A majority of the experimentally employed radioelements are bone-seekers and induce tumours of the skeleton; polonium, which is taken up by reticular tissue, produces an increase in soft tissue tumours generally; insoluble compounds may act locally, as at the injection site, in the intestinal tract, or in lung. Indirect mechanisms are concerned in the induction of pituitary tumours by radioiodine and of leukaemias by isotopes irradiating the blood-forming tissues generally.

The carcinogenic dose was evaluated in experiments with mice employing radiostrontium. The incidence of induced bone tumours is reduced as smaller doses are administered, and approaches the level of the control population where the Sr^{89} administered is between 1 and 5 μc/kg. At very high dosages, the animals die of subacute effects before tumours appear. Comparison of single with fractionated doses shows that the latter are additive in the middle of the dosage range, more than additive above this point, and less than additive at lower dosages. This is susceptible to interpretation on the basis that the most heavily irradiated cells are killed, while neoplastic changes result from less irradiation.

The latency of the response is difficult to understand clearly; it may well be that it is independent of dose, and it appears to be independent of age of the animal. It is apparently longer in longer-lived animals, but not in direct proportion to the life span. Relative effectiveness of various bone-seeking radioelements depends on many factors whose role is not well understood.

The only data on this subject allowing a comparison between animals and man are from cases of human radium toxicity, discussed by HASTERLIK(20). This element was used widely in clinical practice before 1913 and until about 1930. The best controlled material is a group of patients given pure radium chloride intravenously from 1930 to 1932 in known amounts, who were studied intensively at that time and again recently. The 23 year average retention is 0·6% of the original dose. Skeletal changes at this time range from minimal lesions in the skull and shafts of the long bones to gross aseptic necrosis of bone. Retention follows the square root of time in man; in the dog it follows a power function with a higher exponent. Minimal radiological lesions appear at a retained burden of 0·4 μg and a sarcoma appeared at 23 years after injection in a patient with a burden of 3 μg. In other reported cases including dial painters there is uncertainty as to the purity of the material; an important consideration in view of the greater toxicity of mesothorium and radiothorium. It appears that there are still many individuals living with undiscovered radium burdens, including some who never knew they were receiving radium injections.

HARRISON, RAYMOND & TRETHEWAY(21) made use of slow neutron activation analysis to measure the turnover of strontium and barium in man, by administering the stable elements and inducing in the excreta the respective 1·4 hour barium-139 and 2·7 hour strontium-87. After intravenous injection, the elimination is almost wholly by the kidney, while after ingestion the bulk is lost in the faeces. The absorption of calcium:strontium:barium is in the ratio of 10:5:1 indicating a physiological barrier to absorption of the latter two.

3. EFFECTS ON REPRODUCTIVE SYSTEM AND ON FOETUS

RUSSELL & RUSSELL(22) described the effects on the mouse foetus of X-irradiation *in utero*. A single dose of 200 r delivered during the preimplantation period results in a high incidence (80%) of prenatal death. The same amount during the period of major organogenesis (roughly the second week) causes one or more visible malformations in all of the offspring, associated with a considerable proportion of neonatal deaths. During later foetal life these abnormalities fall to low levels, although others have noted damage dependent on destruction of cells in a particular state of differentiation.

It is always noteworthy that a particular skeletal abnormality is produced by an irradiation at a particular time, usually within a one or two day period. This critical period is not obviously related to a particular state of the primordium of the malformed organ. Increasing the dose increases incidence and degree, and also the duration of the critical period; and different abnormalities show different relations of dose to incidence (for many there appears to be a threshold). Some are produced measurably at 50 r, and some changes not classed as abnormalities are seen at 25 r. In the human being this critical period corresponds to about the second to seventh weeks; the writers suggest that pelvic irradiation of women of childbearing age might therefore be wisely limited to the first two weeks following the menses.

CASARETT & HURSH(23) described results of experiments examining spermatogenesis and testing fertility of beagle dogs given low doses of total body X-ray, 5 days a week, throughout life. Dosages were 0·3, 0·6, and 3 r per week to maximum accumulated doses of 62, 117, and 528 r respectively. The two lower dosage groups (permissible level and double that rate) show a very doubtful threshold effect which also appeared in a control. At 3 r per week the sperm counts declined progressively to 10% of controls while 60–180 r were being accumulated; concomitantly, abnormal and dead sperm were found and most of the dogs proved to be infertile. These results seem to confirm the assumptions on which the present permissible level of external radiation is based.

FITZ-NIGGLI(24) investigated the hatchability of eggs fertilized by sperm of Drosophila males previously treated with X-ray (3 000 r), and correlated this with histologic changes in the males. It appears clear that hatchability is lowest (around 10%) between 7 and 10 days after irradiation. In combination with the histologic findings it is concluded that the greatest sensitivity is in the maturing spermatids and the period of their transformation to spermatozoa. It appears that dominant lethal mutations and the formation of abnormal sperm unable to inseminate are both factors in decreased hatching. Spermotocytes and spermatogonia are resistant. 31 MeV X-rays are measurably less efficient than 180 keV X-rays.

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GENETICS — FUNDAMENTAL EFFECTS OF RADIATIONS AND THE HAZARD TO MEN

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Abstract—Four of the papers offered at Geneva were concerned with the genetic hazard to man of ionizing radiations. H. J. MULLER's paper "How Radiations Change the Genetic Constitution" reviews the action of ionizing radiations on the genetic material; summarizes the fundamentals of population genetic theory; and attempts an assessment of the effect on a human population of an increased mutation rate, drawing on data from animal experiments for estimates of some of the genetic parameters. W. L. RUSSELL's paper "Genetic Effects of Radiation in Mice and their Bearing on the Estimation of Human Hazards" describes some of these experiments, notably one in which the relationship between mutation rate and radiation dose was measured for seven gene loci in mouse spermatogonia. T. C. CARTER, in his paper "The Genetic Problem of Irradiated Human Populations", questions the extent of applicability of current genetic theory to human populations. BRUCE WALLACE, in his paper "The Genetic Structure of Mendelian Populations and its Bearing on Radiation Genetics", emphasizes the importance in *Drosophila* populations of mutually cooperating blocks of coadapted genes with a selective advantage in the heterozygous state, and suggests that they may be important in human populations also.

FOUR of the papers offered for presentation at the Geneva conference were concerned, directly or indirectly, with the genetical results of exposing human populations to ionizing radiations. All four accepted it as axiomatic that man does not differ in principle from other organisms in his reactions to ionizing radiations, and that any additional exposure will have genetical consequences. All accepted it as overwhelmingly probable that some, at least, of the consequences will be harmful to individuals. All agreed that exposure at low dose rates, such as to widespread but slight radioactive contamination or to imperfectly shielded X-ray apparatus, if continued over the generations, is potentially more dangerous to a population than exposure to a single flash dose. In all four it was implicit that one of the major tasks now facing the geneticist is to attempt some quantitative assessment of the genetical consequences to man of modern exposure to ionizing radiations. All agreed that this is an exceptionally difficult task, one which is impossible at present with any degree of accuracy, and one which will become possible only after a great amount of research has been completed.

From behind this measure of agreement, however, there emerged disagreement about the method of approach to the problem of quantitative assessment. H. J. MULLER, in his paper, "How Radiation Changes the Genetic Constitution", exemplified what may be called the "gene-summation" approach. This rests on the assumption that human populations are in a state of dynamic equilibrium; then for every mutant gene arising by new mutation there must be, on the average, another which is lost to the population through the "genetic death" of some individual, i.e. through his failure to reproduce, whether because of early death or for some other reason. It is therefore necessary to know only the average rate at which each mutant gene arises by mutation, and the total number of gene loci per individual, to obtain an estimate of the rate at which mutant genes must be eliminated by genetic death. On this basis he attempts a rough quantitive assessment. W. L. RUSSELL's paper, "Genetic Effects of Radiation in Mice and their Bearing on the Estimation of Human Hazards", describes an experimental measurement of one of the parameters on which assessment of human hazards by the gene-summation method rests, viz. the mean induced mutation rate per roentgen per locus. In the absence of a direct measurement in man it is necessary to use the next best thing, viz. measurement on some other mammal. The mouse is probably the only mammal which is small enough, which breeds fast enough, and which is genetically well enough known for such work.

The other two papers in this group, T. C. CARTER's "The Genetic Problem of Irradiated Human Populations" and BRUCE WALLACE's "The Genetic Structure of Mendelian Populations and its Bearing on Radiation Problems", strike a rather different note. CARTER considers the gene-summation approach, accepts it as relevant to grossly deleterious mutations such as epiloia and haemophilia, but doubts its validity when applied to minor genes, which may have complex interactions leading to phenotypes in the normal range. He draws the conclusion that it is premature to attempt any quantitative assessment of the long-term genetic hazard to man; it must be preceded by a massive research effort concerned not only with spontaneous and induced mutation, but also with population structure, including the structure of human populations. BRUCE WALLACE describes some of the effects of gene and chromosome interactions in *Drosophila* populations; sets of genes frequently become "coadapted", and may be beneficial in one genetic milieu though deleterious or even lethal in another. Genes, or block of genes, which are advantageous when in the heterozygous state, relative to both homozygotes, will spread through a population and come into a state of balanced polymorphism in which the gene frequencies are almost independent of mutation rate. WALLACE believes that such genes may be common in man.

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H. J. MULLER's paper is reproduced here in full. Before considering the hazard to human populations, he first describes the types of genetic changes which may be induced by ionizing radiations.

"The changes in the genetic constitution produced by ionizing radiation may for convenience be classified into two major groups, namely, *chromosome aberrations* and *point mutations*.

"The chromosome aberrations consist of losses and additions of whole chromosomes or chromosome parts and/or alterations, called structural changes, in the alignment of chromosome parts. Structural changes are caused by the breakage of one or more chromosomes at two or more points, followed by the junction of the fragments at their broken ends, so as to form a new arrangement, that is, a new linear sequence of their component hereditary particles or genes.(1)

"Point mutations are changes confined to regions of the chromosomes so small that no loss or addition or change in arrangement of genes can be demonstrated by microscopic examinations or breeding tests. Since structural changes range from 'gross' to those so minute as to be at the limit of being detectable as such, there are doubtless other cases of substantially the same kind, but below that limit of size, which become included among the point mutations. However, there is reason to infer that many of the point mutations produced in animals by radiation are not of this kind, but involve changes within the individual genes, and are therefore to be considered as 'gene mutations'. By this it is meant that these changes are restricted to genetic elements too small to be divided either by the process of normal hereditary recombination (crossing over) or by that of gross structural change. This seems to be true also of the great majority of the genetic differences that exist naturally between individuals of the same species, that is, they appear to have arisen as gene mutations.

CHROMOSOME ABERRATIONS

"The chromosome aberrations produced by radiation in the cells of somatic tissues that replenish themselves by proliferation cause necrosis in much of the tissue descended from these cells and abnormality in much of the surviving descendant tissue. This constitutes a major source of delayed radiation damage, some of it never repaired, in the exposed individual himself. The same series of events, occurring among the immature germ cells of the exposed individual, can result in his or her partial or complete sterility. Among mature and nearly mature germ cells, especially spermatozoa, there is a much higher incidence of induction of these chromosome changes, for any given dose of radiation, than among immature germ cells or somatic cells.* Recent evidence(2) confirms the inference(1) that this peculiarity depends upon the chromosomes being in a condensed (tightly spiralized) condition and that it therefore applies also to cells that are in mitosis at the time of irradiation.

"Mature sperm or eggs in which chromosome aberrations (actual or potential) have been induced, function in fertilization, but many of the resulting embryos die in consequence of their abnormal chromosome content. Other embryos, in which there has been gross structural change without excess or deficiency of chromosome parts, develop into normal adult individuals. However, when these seeming normals reproduce, recombination occurs between the structurally

* However, some immature germ cell stages are much more susceptible to chromosome alteration than they appear to be when judged by the frequency with which such alterations are found later, on analysis of offspring derived from the cells that had been exposed while immature. This is because the descendant cells derived from those immature germ cells in which the chromosomes had been altered so often die out, and have their places taken by compensatory multiplication of descendant cells derived from those immature germ cells in which the chromosomes had not been altered. This consideration does not apply in the case of point mutations. (Note added Oct. 1, 1955.)

changed chromosomes derived from one parent and their normal homologues derived from the other parent. In consequence of the non-matching linear arrangements of the genes from the two parents, about 50% of the germ cells now produced have excesses and/or deficiencies of chromosome content. These germ cells usually function, but give rise to embryos (of the second generation after exposure) which die *in utero* at an early stage.(3) This mortality of embryos tends to be repeated over an indefinitely long series of generations. For half of the surviving embryos of such a line of descent, although not themselves containing the lethal excesses or deficiencies, have the grossly changed linear arrangement of genes that, by recombination, again gives rise to these effects.

"In modern human populations there is a tendency to compensate or even overcompensate for reductions in the frequency of viable births, by purposely increasing the number of pregnancies.(4) Hence damage of this kind, once induced, does not tend to die out rapidly but may even spread.

"Fortunately, there are several factors which serve to limit the frequency with which these cases of inherited abortions are produced. One is the fact that the period spent by male germ cells in a mature or nearly mature state averages, at the very most, a few months, whereas they usually spend some 25 years or more—well over 100 times as long—as immature germ cells, relatively insusceptible to the induction of chromosome aberrations. Although the relative lengths of the corresponding periods for female germ cells are not well established the germ cells are, even when nearly mature, much less susceptible than spermatozoa to the induction of the gross aberrations that cause inherited abortion.(5) It may be concluded that more than 99% of the germ cells which function after a given exposure of limited duration (comprising only a few days or weeks) were at the time of that exposure in an immature stage, relatively insusceptible to the induction of chromosome aberrations. In them, aberrations of all kinds were induced with far lower frequency than point mutations.

"Even in the less than one per cent of germ cells that are exposed to radiation of β or γ type during their susceptible stage, gross structural changes of chromosomes will be produced at a low frequency, relatively to point mutations, unless the total dose of radiation received in that period is fairly high, of the order of a hundred or more r units. This is because the production of these aberrations requires at least two chromosome breaks, and these are usually produced independently, by the tracks of different fast particles. On account of being in this sense double or multiple events, these aberrations vary in their frequency according to an exponent of the dose of radiation higher than one (commonly about 1·5).(1) On the other hand, the point mutations vary as single events according to the dose itself. Thus, as the dose is diminished, they do not drop off as fast as the structural changes do, and the latter become rare, relatively to the former.

"It follows from the above considerations that inherited abortion caused by structural change is a relatively insignificant danger even in the case of a large dose of β- or γ-radiation if that has been received in small fractions of not more than a few r per month. If the amount received in any month is higher than this, however, measures should be taken to avoid this damage.

These measures would consist in the prevention or avoidance of conception until the passage of several months after the high exposure. With a very high dose, however, all but the first month of this period would be sterile anyway.

"When the exposure has been to α- or neutron radiation, the production of gross structural changes tends to vary with the dose itself instead of with a higher exponent.(6) This is because both the breaks participating in such an aberration are usually produced by activations arising from the track of the same fast atomic nucleus. In consequence both of this proportionality of the frequency of structural change to dose, with this type of radiation, and of the fact that the more densely crowded activations from such radiation are actually more efficient in breaking the chromosomes, much lower doses, in reps, of neutrons or α-rays than of γ-, β-, or X-rays give significant numbers of structural changes. Hence the rule of not reproducing within some months after exposure should be applied in the case of much lower doses when the radiation has been of these types. In order to gauge how low this limit should be placed, it should be taken into consideration that even 5 reps of neutrons, applied to spermatozoa, may be estimated to induce inherited abortion, based on gross structural change, in some one to six among every thousand viable individuals derived from these spermatozoa.(7)

"The frequency of natural occurrence of gross structural changes giving inherited abortion has not been studied extensively in mammalian populations, but it is known to be low. The highest recorded figure(8) is about 6%. Among offspring from spermatozoa treated with 500 r X-rays, 25% have been reported by two observers.(9)

CHARACTERISTICS OF NATURAL POINT MUTATIONS

"Among the genetic changes induced by exposure to radiation from artificial sources the point mutations are far more frequent and significant than the chromosome aberrations. Among the genetic changes that arise from natural causes (those somewhat misleadingly referred to as 'spontaneous') the point mutations are still more frequent and important, as compared with the chromosome aberrations. Any ordinary population contains a large accumulation, or 'load', of these natural point mutations, which have arisen in the course of many past generations. If any new point mutations are induced by radiation these are added to this already existing load of mutations. They thereupon become lost to view among the latter, in the sense that, with rare exceptions, the origin of any individual point mutations cannot be traced to the radiation. Thus, in order that radiation mutations may be viewed in due perspective, certain salient facts about the natural mutations should first be passed in review.

"Natural point mutations occur sporadically. They are not individually controllable. Any such mutation may be thought of as resulting from an accidental ultramicroscopic encounter between a gene and some atom-group, particle, or photon to which the gene happens, under the circumstances, to be vulnerable. It is probable that, on occasion, instead of the original or 'mother'-gene becoming altered, the accident causes a mis-step in the construction of the 'daughter'-gene, but the effect is much the same as if the old gene had itself mutated. In either case, point-mutational changes are permanent. This implies that the changed gene tends to be very stable, as the original gene was, and that in reproducing it continues to give rise to daughter genes like itself, that is, in this case, of the new type. Thus it 'copies itself' through an indefinite succession of generations.(10)

"The frequency of mutations in general is influenced, however, by many conditions. Thus, cells in certain developmental stages have mutations occurring more frequently in them, in other stages less frequently.(11) There is some evidence that markedly detrimental disturbances in the cellular biochemistry, of whatever nature, tend to favour the occurrence of mutations, while the functioning of the cell within its normal range is associated with a low mutation frequency. Certain special substances, such as the mustard gas series, some organic peroxides and epoxides, and triazine, are so conducive to mutation that they have been termed 'mutagens'.(12) Some of them can in fact be used to induce mutations at about as high a frequency as with radiation. When the distribution of relative frequencies of the different types of mutations induced by one mutagenic agent is compared with that induced by another, or with that of spontaneous mutations, considerable differences are often found, even though most types of mutations produced by one agent are also produced to some extent by any other, and also arise spontaneously but at a lower rate.(13)

"The partial selectivity of action of mutagens does not give evidence of being of such a nature as to result in the mutations produced by a given agent, or under given conditions, being better adapted, as a group, for life in the presence of that agent, or under those conditions, than are the mutations which arise under other circumstances. That is, mutations arising independently of radiation like those produced by radiation are, so far as the organism is concerned, accidents, not adaptive responses. There is evidence indicating that the organism has, through a long period of evolution, been selected for the maintenance of biochemical operations which give it as low a frequency of 'natural' mutations as can practicably be attained, just as it has been also selected to react in such ways as to minimize the occurrence of other accidents.(14)

"It is entirely in line with the accidental nature of natural mutations that extensive tests have agreed in showing the vast majority of them to be detrimental to the organism in its job of surviving and reproducing, just as changes accidentally introduced into any artificial mechanism are predominantly harmful to its useful operation.(10) According to the conception of evolution based on the studies of modern genetics, the whole organism has its basis in its genes. Of these there are thousands of different kinds, interacting with great nicety in the production and maintenance of the complicated organization of the given type of organism. Accordingly, by the mutation of one of these genes or another, in one way or another, any component structure or function, and in many cases combinations of these components, may become diversely altered. Yet in all except very rare cases the change will be disadvantageous, involving an impairment of function.

"It is nevertheless to be inferred that all the superbly interadapted genes of any present-day organism arose through just this process of accidental natural mutation. This could take place only because of the Darwinian principle of natural selection, applying to the genes. That is, on the rare occasions when an accidental mutation did happen to effect an advantageous change, the resultant individual, just because it was aided by that mutation, tended to multiply more than the others. By the continuance and repetition of this process, the type that had been normal became supplanted by other types, that were at least better adapted for life in certain particular environments, or in certain ways. Thus the mutant gene of the previous era became the normal gene of today, and the whole system of genes of the species tended to become ever more differentiated and highly organized. Yet at each stage the great majority of new mutations, if examined before being put through the sieve of selection, must have been detrimental to life or to reproduction, as they are today in all species studied, no matter what the degree of advancement of the species.

"As important for the survival of a species as the differential multiplication of the few better adapted mutants is the reduction in number and eventual dying out, in competition with the 'normal' type, of the much more numerous mutants that are less fit than the normals. Since each generation supplies a fresh crop of these mutations, to be added to those inherited from earlier generations, it is obvious that without this negative selection the system of genes would undergo continued decay. Thus after a time it would become completely heterogeneous, disorganized, and degenerate.(10) In the past, only natural selection has saved it. This selection makes it practically inevitable that any detrimental mutation, no matter how small its harmful effect, will in the long run become eliminated, by tipping the scales against some descendant who carries it, causing his premature death or failure to reproduce.

"However, this dying out of the unfit mutants is in most cases rather long delayed. One reason for this delay is the fact that mutant genes are in the great majority of cases heterozygous, that is, present in individuals which have received the corresponding normal gene from their other parent, and that in such a situation the normal gene usually produces most of the effect. The normal gene is for this reason said to be 'dominant', and the mutant gene 'recessive', even though the mutant is seldom completely without expression when heterozygous.

"Another reason for the delay in the dying out of mutant genes lies in the fact that even in those relatively rare individuals which are 'homozygous' for a given mutant gene, by reason of having inherited that same gene from both parents, the amount of abnormality is often not very great. Hence even in this situation the gene usually confers a much less than 100% risk of premature death, or of failure to reproduce. It may be noted in this connection that the idea that most mutations are monstrosities or freaks is a popular misconception. In fact, only a tiny minority of mutations cause very conspicuous visible abnormalities.

CALCULATION OF NATURAL MUTATIONS PRESENT IN A POPULATION

"The total number of point mutations (or, more correctly, of point-mutant genetic conditions) present in any population at a given time is a product of two interacting numerical factors. The first factor, a, is the total number of new point mutations that arise in one generation. The second factor, b, to be multiplied by the first, is termed the persistence. It represents the total number of individuals of successive generations by whom, on the average, any given mutation, present at first in one individual, comes to be inherited.(15) This same relation holds for mutations of any particular type as well as for the totality of mutations.

"Obviously b, the persistence, depends upon the ability of the individuals carrying the mutation to live and breed, as compared with normal individuals. If for simplicity we assume the whole population to be of stable size, then b, for the average mutation, or for any given type of mutation, is the reciprocal of c, the average chance that an individual who has inherited it will be killed prematurely, or will fail to reproduce, as a result of the one or more functional impairments occasioned in him by that mutation. In getting this average chance of elimination, c, we must estimate the relative frequencies of individuals heterozygous and homozygous for the mutation, and multiply the chance of elimination of each of these types, taken separately, by its relative frequency. When this is done it is found that usually, despite the much smaller detrimental effect in the heterozygous individuals, their relatively large numbers cause most of the eliminations, and most of the total genetic damage to the population, to occur in this group. Thus, in most cases, the homozygous group can for practical purposes be ignored.(15)

"In order to apply this method of calculation to human populations we must first have estimates of a and b. At present such estimates are very indirect, and serve only to indicate a broad range, within which, somewhere, the actual value is probably located. The fruit fly *Drosophila* has thus far been the only organism in which anything like a direct approach has been made to an observed value for either a or b, and even here the results are subject to very large errors. In this material it can be estimated that, in a population of a hundred million, a, the number of new mutations arising naturally per generation that become transmitted to the next generation, is on the average at least eight million, and that b, the persistence or average number of individuals of successive generations which finally come to inherit any given mutation, is considerably more than 20 and probably more than 40. This makes ab, the number of mutations carried by the population of a hundred million in any given generation, probably more than 320 000 000, that is, probably more than three per individual.

"The estimate of a for *Drosophila* was obtained by first taking the observed frequency, 0·18%, with which 'recessive' fully lethal mutations (those that invariably kill homozygous individuals) usually arise in the X chromosome per germ cell per generation when no mutagenic treatment is used.(11) This figure was then multiplied by 6, the ratio of recessive lethals in all the chromosomes to those in the X chromosome. This figure had to be obtained from experiments in which radiation was applied to spermatozoa.(15) The product, 1·08%, representing all lethals, was in turn multiplied by 4, the ratio which all mutations detrimental enough to have been detected by a given technique were found to bear to fully lethal mutations. This figure 4 also was based on radiation mutations.(17) Finally, the second product, 4·3%, was multiplied by 2, because each individual results from two germ cells, and the resultant per cent, 8·6, was multiplied by 100 000 000, the number assumed to exist in the population.

"That the application of the ratio 6, derived from radiation work, to natural mutations is legitimate has been shown by special tests. However, among natural mutations as a group, the ratio of all mutations to lethals is probably a good deal higher than 4, the ratio found among mutations produced by irradiating spermatozoa. For the radiation mutations include a greater proportion of structural changes and these are more often lethal. This is one reason why the final figure for *a* is very conservative. The other reason is that the methods of detection used failed to find mutations that produced less than about 10% risk of premature death, even if they caused considerable infertility, and such mutations may have been relatively numerous.

"The figure for *b* is based on tests, carried out independently by two groups of investigators,(15, 18) to determine how much risk of premature death is conferred by a 'recessive' lethal mutation when it is heterozygous. In both cases an average figure of about 3% to 5% risk of death was obtained. This would result in only one heterozygous individual among some 25 being killed and would hence allow the average lethal a persistence of 25. That is, it would tend to be passed on to some 25 individuals, on the average, before it died out. Since, however, a considerable majority of mutations are not so detrimental as to be fully lethal when homozygous, and most of them are probably not even 50% lethal when in that condition, the figure of 1 in 25 (4%) for the risk of death when heterozygous must be considerably higher than that holding for the average mutation, and the persistence, being the reciprocal of this, would be considerably higher than 25. That is why 40 was used as a better guess for *b*, but the observed distribution of mortalities indicates that even it is likely to be too low.

"Before we can convert our figure *a* for newly arising mutations in *Drosophila* into a corresponding figure *a* for man we must obtain some indication of the ratio of mutation frequency in *Drosophila* to that in man. As yet the only line of approach to this problem lies in a comparison of the frequencies, in the two species, of natural mutations that produce certain specific effects, and that may be inferred to occur at given highly limited positions in the chromosomes. Although the evidence of this kind is meagre and imperfect, there is enough of it to show that in *Drosophila* a mutation of any one specific type, located in a specific chromosomal position (so as to give rise to what is technically known as an 'allele' or 'pseudo-allele' of some pre-existing mutation) arises, on the average, with a natural frequency of between 1 in 100 000 and 1 in 300 000 germ cells, the most likely figure being about 1 in 200 000.(19) In mice there is little published data of this kind as yet, but it would indicate a figure in the range between 1 in 40 000 and 1 in 400 000 or, most likely, about 1 in 140 000.(20) In man, an estimate of between 1 in 50 000 and 1 in 100 000 has been arrived at, on the basis of a much larger amount of data than in either mice or *Drosophila*, but the uncertainties of the methods used in man are much greater.(21) These apparent differences in mutation frequency between the three species may well correspond to the different numbers of cell divisions which take place in their respective reproductive cycles, since these numbers for flies, mice, and men are related about as 1:1·5:2. At any rate, it is likely that the average frequency of mutations of any specific type in man is higher than in *Drosophila*, probably from 2 to 4 times as high. To be conservative we will adopt the lower figure, 2.

"It is, however, likely that the ratio of frequencies of specific mutations in man to those in the fly would not be nearly as high as the ratio of total mutation frequencies in man to those in the fly. For man, and mammals in general, give evidence of having a more complicated organization, all told, than the fly, especially when the complications of the nervous system are taken into account. Mammals may therefore be expected to have a more complex germ plasm than flies, one in which a larger number of different kinds of mutations of specific types can occur. This agrees with existence of a larger amount of the genetic substance, polymerized deoxyribonucleic acid, in mammalian than in fly chromosome sets. Therefore we are in all probability obtaining a low minimal figure for *a* in man if we multiply the *Drosophila a* by only 2.

"For the value of *b* in man or other mammals there is as yet little basis for a decision. The existing indications point strongly to the conclusion that natural mutations in mammals in general, including man, are, as in *Drosophila*, prevailingly recessive, yet not completely so. Moreover, they certainly include a fairly abundant group of 'recessive' lethals, but it is probable that mutations having a lesser degree of detriment are more frequent than lethals. At this preliminary stage of our knowledge of the subject, then, we have little ground for using a markedly different value of *b* for mammals than for *Drosophila*.

"The figure of about 6·5 is thereby arrived at as a minimal one for the content of recessive, definitely detrimental mutations (including lethals), per individual human being. In a preliminary calculation using related methods, the figure 8 was arrived at.(15) These estimates can, as recently shown by SLATIS,(22) be checked in a more direct way. The method consists in observations of the frequency with which homozygous individuals, showing the more definite abnormality often associated with a homozygous mutation, appear among the offspring of marriages between close relatives. Application of this technique has led SLATIS, very tentatively as yet, to the figure 8 as the most probable present approximation to the number of natural mutations of the kind in question for which a person is, on the average, heterozygous. This method now needs to be applied on a much larger scale but the present result is enough to be reassuring, in indicating that our mode of calculation is giving figures of the right order of magnitude.

"It should be emphasized that in these calculations we are dealing only with those mutations which are detrimental enough to give a 'sizeable' risk of genetic extinction by way of premature death, that is, one as great as about 0·5% in the case of the heterozygous individual, or 10% in the case of the homozygous one. We do not know how many mutations arise which are less harmful than this, or which cause extinction mainly by their interference with reproduction, not with life itself. However, even if there are relatively few, those few which have an average grade of detriment within the same order of magnitude as the frequency of their origination by mutation, will accumulate so as to be inordinately numerous in the population. They will provide a very considerable proportion of the superficially observable genetic variability. Moreover, the frequency of the different types of mutations of this group will differ greatly from region to region, in response to differences in the conditions of selection, as well as to random influences.

"Since the frequency with which mutant genes of any given degree of detrimental effect exist in the population at any one time is the product *ab*, where *b* is inversely proportional to *c*, the degree of detrimental effect, it is evident that the existing mutant genes have a distribution, with respect to their harmfulness, very different from the distribution to be found on examining mutations as they arise. For, among the mutant genes as they exist in the population as compared with them at their origination, the less harmful ones are (in inverse proportion to their harmful effect) more numerous than the more harmful ones. For that very reason each slightly harmful mutation that arises tends to cause as much detriment to the population as a whole in the end as each drastically harmful or lethal mutation does, since it compensates for its relatively small degree of harm by afflicting correspondingly more individuals. In consequence, the total amount of genetic damage done to a population by mutations is much more closely proportionate to the total frequency of mutations arising per generation (a/N, where N is the number of individuals in the population) than to the frequency of mutations existing in the population (ab/N).(23) If *a* is raised or lowered, however, it may take scores of generations before its changed value becomes proportionately reflected in the altered average fitness or mutational load of the population. A similar lag occurs if *b* is altered, as happens when the rigor of selection is increased or decreased.

CHARACTERISTICS OF POINT MUTATIONS PRODUCED BY RADIATION

"In the plant material studied by STADLER,(24) evidence was obtained, based on the intensive study of a few types of mutations, that the great majority of apparent point mutations induced by radiation probably consisted of losses of a small section of a chromosome including more than one gene, unlike what was usually true of the natural mutations. In the animal material best studied with reference to this question, that of *Drosophila*,(25) there is evidence that such 'sectional deficiencies' do comprise a good deal larger proportion of the point mutations obtained by irradiation of the mature germ cells than of the point mutations arising naturally. However, the apparent point mutations produced by irradiation of immature germ cells of *Drosophila* do not include substantially more that on further analysis prove to be demonstrable 'sectional deficiencies' than are found among the natural mutations. Moreover, the characteristics of the effects produced on the individual, both in *Drosophila* and mice,(26, 9) also indicate that a large proportion of these radiation-mutations are as truly changes within the genes as are the mutations of natural origin.

"In general then, in the animal material, the radiation-mutations strongly resemble the natural ones. Practically all types of natural point mutations that have been looked for in extensive irradiation experiments have been found to be produced by radiation also. Like natural mutations, of course, the great majority, although not quite all, of those produced by radiation, are detrimental. Moreover, the great majority have far less dominance (i.e.

less expression in the heterozygous individual) than the normal genes from which they arose. Once arisen, the radiation mutations, like the natural ones, are permanent, reproducing themselves as such.⁽²⁷⁾

"Just which mutation is produced by radiation on a given occasion is of course a matter of 'accident', as is true of natural mutations. However, the total frequency of the mutations produced by a given dose of radiation varies to some extent with the accompanying conditions, as in the case of natural mutations, although the conditions in question are to some extent different ones in the two cases. The conditions which influence the production of point mutations by radiation include genetic differences, differences in cell type or stage, differences in metabolic reactions, and (a category overlapping the previous one) differences caused by the application of special chemical or physical treatments.

"For the most part, the same influences have been found to promote or hinder the action of radiation in causing point mutations as in causing structural changes of chromosomes. For example, chromosomes in condensed stages are more susceptible to the induction of changes of both types. Some findings of interesting differences in this respect have been reported, however. Among these are the observations that sperm cells of *Drosophila* several days prior to their release, and therefore perhaps in the spermatic stage, are much more susceptible than mature spermatozoa to the production by radiation of structural changes, but not of point mutations.(28)

"In accordance with the view, first proposed by RAPOPORT(29) on the basis of chemical work by FRICKE,(30) that the mutagenic action of radiation is exerted via the production of actively oxidizing radicals or molecules, it is found that radiation mutagenesis of both major types is positively correlated with the amount of free oxygen present at irradiation. Physical or chemical influences which appear directly or indirectly to increase or decrease the abundance of oxygen available for conversion into mutagenic radicals influence correspondingly the frequency of mutations produced. There is, to be sure, evidence indicating that not all the mutagenic action of radiation takes the same pathway, and that some of it may be quite unconnected with oxidation. But, however that may be, the above and other findings, by demonstrating the conditional nature of radiation mutagenesis, constitute a disproof of the target hypothesis of such mutagenesis, at least in the simplified form in which it had sometimes been applied.(31) Moreover, these findings are of considerable practical value in having led to the working out of treatments, described in other sections of this conference,(32) which give hope of affording significant protection against the mutagenic action of radiation. The fact that certain treatments, even when given after irradiation, aid in such protection, is especially noteworthy, both from a theoretical and from a practical standpoint.

"In material of varied kinds, but more especially in *Drosophila*, there is good evidence that over a considerable range of dose (in *Drosophila*, from some 50 r to more than 1 000 r, a more than 20-fold range) the frequency of point mutations (like that of chromosome breaks) is directly proportional to dose.(33) Moreover, they are independent of the timing of the dose, over an enormous range, provided cellular conditions are held constant.(34) Below 25 or 50 r the mutation frequency is so low that it has hitherto been impossible to obtain sufficient data, and above 1 000 or 2 000 r the determination of frequency may be interfered with by a selective elimination (through chromosome aberrations) of the cells that happened at irradiation to be in a more susceptible state.(35) Since, however, in the work with low doses and low time-rates of delivery of γ-radiation the germ cells of some series were traversed by only one electron track in a period of a half-hour or more, on the average, and still showed a frequency of mutations proportional to the total dose, there is reason to infer that no dose or intensity of such radiation is without its proportionate production of point mutations. Moreover, if this is true of γ-radiation it must be at least as true of radiation producing tracks more densely crowded with ionizations.

"Despite the equal mutagenic efficiency of different doses and dose rates of ionizing radiation, it is not necessary to infer that a point mutation or a break is ordinarily the consequence, direct or indirect, of a single activation or even of a single ionization. For all the ionizing radiation studied has some of its ionizations produced in clusters of minute diameter. If two or more ions commonly co-operate mutagenically, however, it might be thought that this would become evident by causing the frequency of mutations to vary as the square or some higher power of the dose. Yet this would not be true if those ions had to be as near together as the ones in a natural cluster, for such close juxtaposition as this would not be brought about with appreciable frequency by raising the dose and the dose rate within toleration limits.

"That such co-operation in mutagenesis does occur is indicated by recent observations to the effect that fast neutrons appear to be approximately twice as efficient as X- or γ-rays in inducing point mutations in the chromosomes of *Drosophila* spermatozoa,(36) and are probably a good deal more efficient still, relative to X- or γ-rays, in inducing chromosome breaks.(37) Presumably α-rays likewise would be more efficient than X- or γ-rays in these respects. One possible interpretation of this higher effectiveness of fast neutrons would be provided, on the Watson-Crick hypothesis of the structure of the genetic material, by the doubleness of the fibres in which the rearrangements are produced, if we suppose that the occurrence of the mutation or break is much facilitated when both fibres are simultaneously affected.

"The effectiveness of fast neutrons in inducing point mutations is actually higher than it appears to be, because intensive studies of given cases of these seeming point mutations have shown that in fact a considerable proportion of them involve a double or multiple effect within a very localized chromosome region.(38, 39) This greater clustering of effects with neutrons than with X- or γ-rays is to be expected, in view of the greater concentration of the ionizations in the tracks of the ionizing particles released by neutrons, provided that the mutational effects arise in close proximity with the activations that induce them. Since this clustering of effects causes many of them to be lost to view by reason of their proximity to each other (except when special techniques of analysis are used), the mutagenic potentiality of the fast neutrons is correspondingly underrated in most experiments. So far as genetic damage to the population is concerned, however, a double or multiple effect of the given kind adds no more to the mutational load than does a single effect. Hence for present purposes fast neutrons may be regarded as no more than twice as effective as X- or γ-rays in producing point mutations.

ESTIMATION OF THE TOTAL POINT MUTATIONAL DAMAGE FROM A GIVEN AMOUNT OF RADIATION

"It has been noted that the important quantity in the determination of the total amount of genetic damage is not the amount of harm done to the individuals who have inherited the mutations in question but only the total number of these mutations. For a mutation doing less harm to an individual will, as if in compensation, be passed down to a correspondingly larger number of descendant individuals. It has also been noted that an approach to a direct estimation of the total number of mutations arising has thus far been made only in *Drosophila*, and that this calculation has involved the use of data from radiation experiments. This work can therefore be applied to the estimation of the total damage arising from a given dose.

"The principles have already been explained whereby a minimum value for the total number of mutations is obtained by getting the number of lethals in the X-chromosome and then multiplying this by 6, to get the number of lethals in all the chromosomes, and again by 4, to get the total number of mutations causing at least 10% detriment to life, when homozygous. (A correction is made in this calculation, based on certain tests, in order to estimate the number of point mutations without including the structural changes.) When this calculation is carried out, using the results obtained at any given dose, the resulting number can then be expressed in terms of the total number of point mutations produced by a single r unit, by using the principle of proportionality of point-mutation frequency to dose. It is then found that this number turns out to be about 1 mutation among 2 000 germ cells per r (that s, 5×10^{-4}/r) for X- or γ-rays applied in the usual way to mature spermatozoa.⁽⁴⁰⁾ The more important figure, representing the result of irradiation of the more prevalent stages (gonia) of immature germ cells of adult *Drosophila*, is only a fourth to a half of this, according to the conditions. It is probable that there are even lower values for certain other immature stages of *Drosophila* germ cells, as for instance those in the embryonic polar cap.⁽⁴¹⁾

"In order to obtain a figure for the total number of mutations produced by a given dose in mammalian material we may follow the procedure which we adopted in the calculation of natural mutations. This involved a comparison of the mutation frequencies involving particular types of mutations, located in given positions on the chromosome, in *Drosophila* and in mammals, and then applying the ratio thus found to the figure for total mutation frequency in *Drosophila*, so as to convert it into the presumed corresponding value (a minimal one) for mammals.

"Fortunately there is available for this comparison a much more reliable body of data, for both groups of organisms, than that which we had recourse to in the case of natural mutations. The average frequency of point mutations of the kind in question in *Drosophila*, based on a study of ten types ('loci'), was found to be about $1{\cdot}4 \times 10^{-8}$/r for any given type, when the radiation was applied to inactive immature germ cells ('oögonia').⁽⁴²⁾ The different types seldom varied from one another in frequency by a factor of more than 2 in material abundant enough for judging this matter (that in which spermatozoa had been irradiated).

"In the mammalian material, comprising irradiated spermatogonia of mice, RUSSELL(20) has reported an average mutation frequency of about 25×10^{-8}/r, based upon seven specific types of mutations ('loci'). Here the range of variation between the different types was greater than in the above *Drosophila* material, but their mean agreed well with their mode, and four of the seven types conformed fairly closely with this mean. It is clear on comparison of the two sets of results that the susceptibility of the mammalian material is at least an order of magnitude higher than that of the flies, the observed factorial difference in results being 18. To obtain a minimum estimate of the total frequency of mutation in the mice we must therefore multiply by 18 the figure arrived at for gonial cells of flies. (It is of no consequence that in the flies oögonia were studied and in the mice spermatogonia, since special comparisons(43) have shown these two cell types to be alike in mutagenic susceptibility, as they are expected to be.) Since the figure for the gonia of flies had a lower limiting value of $1{\cdot}25 \times 10^{-4}$/r the minimum value for mice becomes $2{\cdot}25 \times 10^{-3}$/r. This is the frequency for a germ cell, not for an offspring derived from two such germ cells; for the offspring it would be $4{\cdot}5 \times 10^{-3}$/r.*

"In performing this calculation we are, as in the case of the natural mutations, assuming that the hereditary material of mammals is no more compound than that of flies, i.e. that there is not a greater number of different specific types of mutations in mammals than in flies, despite their seemingly more complicated organisms and their larger amount of deoxyribonucleic acid. The total frequency of mutations per r may be a good deal higher than here calculated not only because of the inadequacy of this assumption but also because weakly detrimental mutations and those mainly affecting fertility rather than individual survival have not been included. Moreover, only the lower limiting value for the somewhat variable mutation frequency of fly oögonia was used. All this emphasizes the fact that our estimate is decidedly on the 'conservative' side.

"At the same time, it is true that the value is one for mice, not human beings. All that can be said to this is that, so long as we lack data on an organism still closer to man, it is necessary, provisionally, to base our judgments on this result, and that, since mice are so much closer to men than flies are in almost every other important respect, it would be strange if they were not closer in their mutagenic properties as well. Moreover, the factors which might be expected to cause a significant difference in the natural mutation frequencies of mice and men—their great discrepancies in length of life, size, and number of cell generations in the reproductive cycle—would not be expected to exert significant influences on the frequency with which mutations are produced in them by radiation.

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* My estimation of the minimum total rate of mutation in mammals, as derived from the data in mice and in *Drosophila* taken in combination, could be made much more direct, without complicating the picture by considering just how much more sensitive the genetic material of mice is than that of *Drosophila*. The ratio of sensitiveness of the two organisms is subject to a very high error, simply because of fluctuations in, and imperfections in our knowledge concerning, mutation frequencies in gonadal stages in *Drosophila*. This ratio of sensitivities in the two organisms is irrelevant to the question at issue if only we assume that the ratio of mutations at an average specific locus to total mutations is roughly the same (when we confine ourselves to point mutations) for all the stages of a given organism. On this assumption, combined with the assumption that total mutations are at least as frequent in relation to mutations at a specific locus in mice as in flies, all we need to do is to use Russell's figures for the average frequency at a specific locus in mice and multiply it by the ratio of total to specific mutations in *Drosophila*, in order to get the minimum estimate which we are after. (Note added Oct. 1, 1955.)

"The minimum figure of $4{\cdot}5 \times 10^{-3}$/r point mutations for the offspring of parents both of whom were exposed can be expressed in the form: 'at least one induced point mutation per offspring, on the average, for each 220 r of exposure to both parents'. From this it is evident that many of the children who were conceived by Hiroshima survivors at any time after their exposure must have contained one or more mutations induced by the radiation. Similarly, children conceived by parents both of whom have been exposed to the so-called 'permissible dose' of 0·3 r per week (15 r per year) for as long as fifteen years would on the average contain at least one induced mutation. It is probable that the same is also true of the children of many radiologists, dermatologists, and dentists.(44)

"The recent study of MACHT & LAWRENCE(45) gives direct evidence of genetic damage in such cases and is in this respect superior to the studies made in Japan. Moreover, studies of MOELLER *et al.*(46) show that the population in general is already receiving significant amounts of radiation from medical diagnoses. SONNENBLICK(47) finds that exposures of this kind are seldom adequately controlled.

"When it is considered that practically every mutation must eventually become eliminated from the population, after having—even if imperceptibly—hampered enough descendants so as finally to be a deciding cause, in the last of the line, of his premature death or failure to reproduce, then it becomes evident that practically every mutation represents a postponed disaster. Thus the genetic damage, that to later generations, caused by a given total dose is seen to be far greater than the damage to the exposed individual himself. In view of this, measures and regulations concerned with radiation protection should be based, at least in the case of persons who may later reproduce, primarily on the risk of genetic damage or, more specifically, of point mutations in their germ cells, rather than on the risk of damage to their own bodies. This would cause such measures and regulations to be far more stringent than they are at present.(48)

THE INDUCED MUTATIONS IN RELATION TO THE NATURAL LOAD

"On our conservative estimate of 16 000 000 natural mutations arising per generation in a population of 100 000 000, a frequency of 0·16, it would take only about 37 r of γ-radiation delivered to the population to produce a quantity of new mutations equal to the new natural ones, and thus to double the mutation frequency. Our conservative estimate, however, was based on the assumption of only 1×10^{-5} as the average frequency of a mutation of some specific type, involving a given chromosomal position. According to this assumption the actual data, which indicate about 2×10^{-5} as the frequency of mutations of a specific type, are misleadingly high, because of certain sources of technical error. Since, however, this is a matter by no means proved as yet, it remains quite possible that the amount of radiation necessary to double the mutation frequency is 75 r or higher. This is approximately the value that we used in our earlier treatments of the subject,(48, 49) in which it was assumed that the observed 2×10^{-5} frequency for mutations of a specific type was approximately correct. These considerations illustrate the considerable margins of error in any present quantitative treatments, and the need for greater exactitude of knowledge.(50)

"The present uncertainty regarding the natural mutation frequency carries with it a corresponding uncertainty regarding what proportion of the natural mutations in man are contributed by natural radiation. There is also uncertainty regarding this question based on variation in the amount of natural radiation. If we suppose that in some typical regions as much as 6 r are accumulated, on the average, in the span of one human reproductive generation (25 to 30 years), then, on the more conservative estimate that the natural mutation frequency is equal to what would be induced by 37 r, it turns out that some 16% of the natural mutations in man are produced by natural radiation. On the higher estimate for natural mutations, some 8% of them would be radiation-induced. In either case, the figure must be far higher than for short-lived organisms, such as mice or flies. On the other hand, in some human populations living at a high altitude, with its greater cosmic ray intensity, the contribution of radiation to the natural mutation rate must be twice as high as here estimated. Still higher values must obtain for some populations living in regions where radio-active minerals are abundant.

"Many persons unfamiliar with genetics have regarded the seeming normality of the children born to survivors of the Hiroshima and Nagasaki bombings as evidence against the conclusion that the amount of radiation there received produced a significant amount of genetic damage. This misunderstanding arises from their lack of realization of the following points: (i) Few mutations are sufficiently dominant to give readily perceptible effects when inherited from only one parent, as they are in the vast majority of cases. (ii) Even though these effects are not perceptible they are nearly always sufficient to hamper the individual somewhat, and finally, usually in a very distant descendant, to cause the extinction of that line of descent. (iii) In any heterogeneously breeding population, such as is found anywhere outside of the geneticists' fields and laboratories, there is already so much natural genetic variation, representing an accumulation of many generations of natural mutations, that the additional mutations caused by the radiation would become lost to view among them even if they were as abundant as those that would arise naturally in the course of a number of generations. Thus, the genetically damaged population will eventually have to pay the costs, but these will be spread out over so many small instalments, and so intermingled with the greater weight of other payments, as hardly to be recognizable. All this was of course well known to geneticists before the observations on the children at Hiroshima and Nagasaki were conducted, and led them to express serious doubts that any genetic effects would be demonstrable there, even though they had no doubts that they had actually been produced.(40)

"These points may be better appreciated if it is realized that in *Drosophila* also it had not been possible to demonstrate the mutagenic action of radiation by mere inspection of the individuals of the first, second or third generations after exposure. Exact genetic methods had first to be worked out(10) and these are of course unavailable in man. Even following an exposure of fly spermatozoa to some 5 000 r, which we today know causes each offspring, on the average, to receive at least 3 induced mutations, hardly one abnormal offspring is usually to be found among 100 examined, yet the damage is there, and it will be exerted if the population is allowed to continue.

"At the same time, it is true, unlike what many non-geneticists suppose, that the effects of the genetic damage are more strongly exerted in the first generation of offspring than in any subsequent generation. They very gradually subside, in the course of many generations, as the population is purged by the dying out of the unfit. Even the recessive effects, those present in individuals homozygous for the given mutations, are found most frequently in the first generation, and then less and less frequently if the population breeds naturally rather than being subjected to a geneticist's controlled inbreeding manipulations. Moreover, there is a much higher chance that a given induced mutation will become homozygous by meeting, at fertilization, a gene of the same type derived from the great accumulated store of natural mutations, than one of that type which, like itself, had been induced by the radiation.

"Since the worst effects are already exerted in F_1, what the Hiroshima observations do demonstrate clearly is that the genetic damage to posterity caused by exposure to between one hundred and several hundred r is not *conspicuously* detrimental, and is well within limits consistent with the survival and self-perpetuation of the population. This might have been reckoned as probable without the direct evidence. For, according to the conclusion that the average individual is already heterozygous for some 6, 8, or even more mutations which when homozygous would be fairly conspicuous and/or detrimental, it does not seem likely that the addition in heterozygous condition of just one more, induced by some 200 r, would result in any very evident change in the picture. This remains true even when we take into consideration the fact that the already existing mutations have already passed, to varying extents, through the sieve of selection and are therefore not, on the average, as detrimental as the newly induced ones.

"The apparent contradiction between the fact that a really serious amount of genetic damage was produced and the fact that none is evident even in the most afflicted generation (the first), is reconciled by the manner in which the damage is spread out, thinner and thinner, over a great number of generations. There is a kind of buffering or dilution of the damaging effects, by the normal genes that dominate over them and thus delay their elimination. Thus the effects are spread out in time, in inverse proportion to their dilution in any one generation, but the total damage remains just as great as if concentrated. Moreover, even though the induced mutations may be many times the number that would arise naturally in only one generation, they are nevertheless few in relation to the accumulated natural 'load'. Hence they can raise by only a rather small per cent the number of genetic shortcomings already present in the population.

"Despite these buffering influences, it would be impossible for a population to tolerate, generation after generation, an exposure which, given to only one generation, would cause no perceptible deterioration. Gradually, as elimination rose enough to balance the new mutations, an equilibrium level of accumulation would be approached, and at this new level the then existing accumulated load would be as many times greater than the original accumulated load as the then existing mutation rate, a population which had received this dose for many generations would at last have twice as many ills of genetic origin as we have. Yet we already have more than enough for comfort.

"Not to be neglected in the picture is the other end of the balance mechanism: the rate at which elimination of mutations goes on. Under modern civilization we interfere so much with this that we are probably raising the load of accumulated mutations as fast as by applying some tens of r to every one's reproductive organs.(44) Under these circumstances the raising of mutation frequency at the same time, by exposure to radiation, might tend to bring us to a genetic situation that it would be difficult to cope with.

"All these questions need to be not only discussed but actually investigated far more realistically than they have been in the past. Otherwise we may at last find ourselves, genetically, facing a parallel to already accomplished deforestation and erosion, on an even grander scale. This problem is not only one that is concerned with the possible aftermaths of atomic war. It must be faced equally by the proponents of peace if we are to have an atomic age, with its risks of prolonged 'permissible' exposures arising from industrial uses and radio-active waste products.

"For peace will, we hope, go on and on through a great series of generations. Under these circumstances, it will be the more necessary to control and limit the radiation received by the population at large in every generation. For, given enough generations, the equilibrium level of damage will be reached, at which that damage will no longer be buffered, but will accurately correspond with the existing mutation frequency. Then, a relatively small number of r per generation will exert an inordinately larger effect than it seems to now. At our present juncture, before that process has more than begun, far-seeing policies should be established. These must guard us against the dangerous fallacy that what cannot be seen or felt need not be bothered with.

"This subject of protection of human beings against the genetic damage produced by radiation must, until suitable policies are established, far overshadow in its importance that of the utilization of radiation in the genetic improvement, for human purposes, of organisms potentially useful to man, or in the elimination or reduction of noxious organisms. However, these constructive uses of radiation in 'biological engineering' will come increasingly to the fore as the more menacing aspects of radiation are brought under control. There is already abundant evidence of the possibility of such beneficial applications on a considerable scale.(51, 40)

"At the same time, the dangerous mistake should not be made of considering man as a species who would himself undergo a long-term benefit from the application of radiation to his germ plasm. His own reproductive material is his most invaluable, irretrievable possession. It is already subject to an amount of variation which, in relation to his present reproductive practices, borders on the excessive. Under these circumstances, man's first concern in dealing with radiation must be his own protection."

* * *

W. L. RUSSELL points out that in man the effects of irradiating spermatogonia are of far greater practical importance than those of irradiating post-spermatogonial stages. The length of time spent by a male germ cell in post-spermatogonial stages is probably a few weeks, or at the most a few months; during the rest of the individual's life it is represented by a spermatogonium.

"Studies on the genetic effects of ionizing radiation in mice conducted over the past few years have made it necessary to reconsider various aspects of the estimation of the genetic hazards of radiation in man. In the space available here, only the general conclusions from these studies can be presented. Most of the work has been done with irradiated males. The limited results so far obtained from irradiation of females will not be discussed here.

"In considering the genetic effects of irradiation it is important to distinguish the stage, or stages, in gametogenesis at which the gametes have been irradiated. This distinction can be made clearly in experimental work with mammals. Male mice exposed to an acute X-ray dose of a few hundred roentgens remain fertile for about four or five weeks. A period of sterility then sets in, after which fertility returns and is maintained. The temporary sterility is due to the depletion of spermatogonia, which are especially sensitive to killing by radiation (OAKBERG, 1955). Fertility returns when adequate repopulation of spermatogonia has occurred. Matings made during the pre-sterile period utilize germ cells that were already in post-spermatogonial stages at the time of irradiation. Matings made after the sterile period utilize germ cells that were in spermatogonial stage at the time of irradiation. The genetic effects of irradiation in these two broad stages, spermatogonial and post-spermatogonial, can, therefore, be measured separately.

"It has long been known, from the pioneer investigations of STRANDSKOV on guinea-pigs and of SNELL and HERTWIG on mice (see review by RUSSELL, 1954), that when the male gametes are heavily X-irradiated in post-spermatogonial stages the offspring show high incidences of dominant defects, such as prenatal lethality, partial sterility, and sterility, that result primarily from major chromosomal aberrations. HERTWIG's work also showed that, in the offspring that resulted from male gametes that had been irradiated in spermatogonial stage, these particular defects were either absent, or greatly reduced in frequency. This finding has been confirmed in extensive experiments in our laboratory.

"In man, the conditions are fortunately such that the radiation dose received by a male germ cell in its post-spermatogonial stages will usually be small compared to that received in spermatogonial stages. For example, even with continuous irradiation at the official maximum permissible level right up to the time of mating, only a small dose is accumulated in the five weeks or so required for development of the spermatozoa from spermatogonia. In exceptional cases, such as accidents or emergencies requiring the taking of unusual risks, the gonads of a man may be exposed to a considerable dose of radiation within a short time. In this event, it is recommended that the individual abstain from procreation for the few weeks required for the disappearance of germ cells that were irradiated in post-spermatogonial stages. Thus, even in these cases, the risk of transmission of mutational changes can be reduced to that incurred from irradiation of spermatogonia. Unfortunately, as will be shown, even this risk is far from negligible.

"Since the practical importance of mutation induction in spermatogonia far outweighs that in post-spermatogonial stages, determinations were made of the X-radiation–induced point mutation rates at seven specific autosomal loci for spermatogonia of mice (RUSSELL, 1951). The mean rate obtained from an experiment in which the males were exposed to a dose of 600 r, was $(25{\cdot}0 \pm 3{\cdot}7) \times 10^{-8}$ per roentgen, per locus. Additional unpublished data from this experiment and from another experiment in which the males were exposed to 300 r, give approximately the same mutation rate per roentgen. This rate is approximately one order of magnitude higher than that found in similar experiments with *Drosophila*. One of the *Drosophila* experiments was designed to parallel the mouse experiments as closely as possible, and was carried out in our laboratory by ALEXANDER (1954). In this experiment the X-ray–induced mean mutation rate for eight specific autosomal loci in *Drosophila* spermatogonia was determined. The mouse induced mutation rate is fifteen times higher than the *Drosophila* mutation rate obtained in this particular experiment. From a scientific point of view, it would be unwise to generalize from experiments involving a limited number of loci. However, since we are concerned with the immediate practical problems of protection in man, it would be risky to ignore the indication that calculations of human hazards based on *Drosophila* mutation rates may seriously underestimate the damage.

"It has sometimes been suggested that the mutation frequency in the offspring of an irradiated individual might decline as the interval between irradiation and conception increased. It has already been shown that there is a decline in frequency of transmitted chromosomal aberrations when the father has exhausted the gametes that were irradiated in post-spermatogonial stages. After the father has reached this state, that is, when all future offspring will come from germ cells that were irradiated as spermatogonia, does the frequency of point mutations transmitted to the offspring decline as the time after irradiation increases? No information from experiments on irradiated *Drosophila* spermatozoa could be considered an answer to this question about spermatogonia. Furthermore, it was desirable to have this particular problem investigated on an organism with a life span considerably longer than that of *Drosophila* and with a testis histologically similar to that of man. Analysis of the data now accumulated on mutations induced at specific loci in spermatogonia of the mouse shows no significant change in mutation rate with time after irradiation. It may be concluded that these data indicate that, in man, offspring conceived long after exposure of the father to radiation are just as likely to inherit induced mutations as those conceived a few weeks after exposure. Putting this in practical terms, the results of the mouse work indicate that, although postponement of procreation for a few weeks following exposure to irradiation would reduce the total risk of transmission of mutational changes, by excluding those induced in post-spermatogonial stages, further postponement would not give any additional reduction in risk.

"In addition to the data on mutation rate, other information obtained from the specific loci experiments on the mouse has a bearing on the estimation of human hazards. One type of information that is important in this respect is the nature of the mutations. More than one-half of the observed mutations induced in mouse spermatogonia have proved to be lethal in homozygous condition. From *Drosophila* results, it might be expected that these would, on the average, have deleterious effects in heterozygous condition. Clear-cut deleterious effects of some of the individual radiation-induced mouse mutations in heterozygous condition have been found. This increases the importance of investigating whether or not significant damage can be found in the first generation descendants of male mice whose gametes have been irradiated in spermatogonial stage.

"The specific loci experiments were not designed for measuring over-all damage in the offspring. However, one possible indication from these experiments that such damage may not be negligible, even when the gametes receive the radiation in spermatogonial stage, is provided by the data on litter size. This was recorded when the litters reached approximately three weeks of age, the age at which they were examined for mutations. In all experiments, the litters sired by irradiated males showed a slightly lower mean litter size than those sired by control males. In the 300 r experiment the reduction was between three and four per cent. It should again be emphasized that all these data were obtained from gametes irradiated in spermatogonial stage. If the effect on litter size really represents a reduction in viability of the offspring, it is quite possible that appreciable effects will be found in experiments now being conducted to measure over-all damage in the first generation descendants of mice whose gametes were irradiated as spermatogonia. In any case, first generation effects, even from irradiated spermatogonia, appear to be important enough to warrant further investigation. Adequate protection against the genetic hazards of peaceful uses of atomic energy may require a limitation not only of the average dose of radiation received by the population as a whole, but also of the dose accumulated by individuals. The magnitude of the first generation effects already observed, with the doses used in these preliminary experiments with mice, indicates that it is quite possible that, if the present permissible weekly dose is to be kept, a total accumulated dose limit may have to be established to protect the individual from incurring too great a risk of damage to his own offspring."

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T. C. CARTER considers the gene-summation method of assessing the genetic hazard to man, and accepts its validity when applied to grossly deleterious major mutants. Applied to all human genes, however, he considers its value doubtful, for two reasons: first, the resulting estimate of genetic damage may vary over such a wide range, according to the assumptions made, as to be of little practical use; second, because the method disregards population structure and gene interactions, and these may be of great importance.

"Attempts to make quantitative estimates of genetic damage to human populations by ionizing radiations depend upon the concept of genetic equilibrium. They usually run on the following general lines. First, an estimate is made of the proportion of all deaths in the unirradiated population which are 'genetic deaths' due to spontaneous mutation. The average mutation rate per locus, m, is estimated and multiplied by an estimate of n, the number of loci in the human genome; the product is an estimate of mn, the average number of newly arisen mutant genes per gamete; $2mn$ is the average number per zygote. It is then assumed that an individual suffers 'genetic death' whenever he carries z mutant genes more than the average; thus $2mnp$ mutant genes would be eliminated through the genetic death of $2mnp/z$ individuals. But $2mnp$ is the number of mutant genes arising by new mutation in p individuals. In other words, among p deaths $2mnp/z$ will be genetic deaths; or a proportion $2mn/z$ of all deaths will represent genetic deaths. The next step is to estimate the factor, y, by which the mutation rate is increased under the given irradiation conditions. When a new genetic equilibrium has been reached, the fraction of all deaths which are genetic deaths becomes $2mny/z$. The radiation-induced genetic deaths are then a fraction $2mn(y - 1)/z$ of all deaths.

"When attempts are made to put numerical values into this expression it becomes apparent that any present-day quantitative estimate of radiation-induced genetic damage to man has extremely tenuous foundations. Numerical estimates vary from 10^{-5} to 10^{-7} mutations per locus for m and from 10 000 to 100 000 loci for n; and there are no human data on which to base estimates of y and z. For several loci in the mouse y probably has a value of about 2 for an accumulated radiation dose of 50 r. In the mouse also it is possible to obtain viable and fertile individuals homozygous for eight major recessive mutants, i.e. carrying 16 major mutant genes, presumably over and above the minor mutants which are present in the average individual; it therefore seems quite possible that in this species z may be greater than 10. If these figures were applicable also to man, it would follow that exposure to chronic irradiation at a dose-rate of 50 r per generation would result in the induction of genetic deaths amounting to somewhere between two tenths and two ten-thousandths of all deaths. Thus the range of uncertainty in such calculations is colossal; and I feel by no means convinced that the truth necessarily lies within even this enormous range.

"The main lesson to be learned from the sort of calculation made above is, I think, that we are profoundly ignorant of all quantitative aspects of this subject; the corollary is that a massive research effort is needed. What form should this take?

"Our final objective is to make accurate quantitative predictions about the effects on human populations of exposing them to chronic dosage with ionizing radiations. It seems to me that a research programme with this end naturally falls into three main parts: research on mutation, since radiations induce mutations; research on animal populations, since man is a social animal; and research on human populations, since one cannot justify the application to one species of quantitative measurements made on another.

"Research on mutation is in progress in genetical institutes throughout the world. It entails a great deal of 'counting heads', and for that reason much of it is done on virus, bacteria and other micro-organisms; enormous numbers of these can be cultured in a small space, and techniques have been developed whereby the identification of mutants becomes a simple routine. Furthermore, in these organisms the final, phenotypic effect of the gene is probably causally close to the primary gene action, a fact which makes it less difficult to investigate the processes involved. The disadvantage of work with micro-organisms lies in the fact that the reproductive nucleus always occurs in the same type of cell; and we know that with higher organisms the mutational properties of a gene may vary with the type of cell in which it is carried. More studies of mutation are needed, but they must be spread over many types of cell in many different organisms, including vertebrates. In particular, we need studies of the effects of ionizing radiations when administered at very low dose rates over a long period, to determine whether the linear accumulative law is really strictly valid under these conditions.

"Experimental research on population genetics has lagged far behind population genetic theory; and anyone who reads the literature in this field cannot avoid a disquietening feeling of doubt about the extent to which theoretical models of populations may bear any relationship to actual populations as they exist in nature. Heterosis, epistasis, linkage, chromosome mutation and assortative mating play a small part, if any, in most population genetic calculations today; yet studies of populations, both in the wild and in the laboratory, suggest that these may be essential elements in population structure. It seems to me that we need a large programme of research on animal population genetics, with the object of determining the factors involved, assessing their relative importance and developing a more adequate population genetic theory. In particular, we need more research on the effects of assortative mating and the extent to which it occurs in wild populations; there can be little doubt that assortative mating with respect to mental characteristics occurs extensively in man. Until we have a clearer picture of what occurs in animal populations, both free-living and experimental, we have little hope of making predictions about human populations.

"The third main field of research should be human genetics, because we cannot make predictions about the changes which will be induced in human populations by additional radiation exposure until we know something about the changes which may occur in its absence. The first thing we need to know is the extent of genetic death in civilized populations and the form it takes. Does it occur through pre-natal inviability, post-natal inviability, susceptibility to infection or accident, inability to attract a mate, organic sterility, disinclination to reproduce? Is it associated with high intelligence or the ability to make significant contributions to civilization? Is it associated especially with mental defect? To what extent does it lead to suffering by the individual concerned, or to his family? To what extent does it place a material load on the rest of the population, e.g. by the upkeep of institutions, hospitals or prisons? To answer these questions we need a study of the mental, physical and demographic characteristics of normal human populations, as they exist today and in the absence of additional radiation exposure.

"Next we need to know the relationship, in man, between radiation exposure and mutation rate. Here two approaches are possible: one is to study small populations which have been exposed to high radiation doses, as at Hiroshima and Nagasaki; the other is to study large populations which are exposed to low radiation doses, e.g. the population of a large area in which the natural radiation background is high. My own view is that the latter is likely to be the more fruitful approach. There are granite areas in Scotland, England, Wales and Scandinavia in which the radiation background is appreciably higher than in the granite-free areas of the same countries; and I think it may be within the bounds of what is technically possible today to make estimates of the mutation rates of a few clear-cut autosomal dominant or sex-linked recessive conditions (e.g. achondroplasia, epiloia, haemophilia) with sufficient accuracy to enable us to set useful fiducial limits to the mutation-rate-doubling radiation dose in man. Such a project would probably have to be on an international scale and would certainly take ten years or more; but I think the results would be well worth the effort."

* * *

BRUCE WALLACE first surveys the development of concepts of the genetical structure of populations. Starting with the genetical counterparts of the taxonomist's type-specimen, viz. an idealized individual in which every gene was a "wild-type" allele, he traces the development of thought to the present-day realization that behind an approximately constant phenotype there may lie an infinite diversity of genotypes. He then considers the impact of this on the assessment of radiation hazards.

"The evaluation of the cumulative effect radiations will have on future generations requires three types of information. It obviously requires an estimation of the amount of radiation to which persons will be exposed. Second, it requires information concerning the genetic effects of these radiations. Finally, it requires knowledge of the genetic structure of populations. The study of the genetic effects, radiation genetics, has since 1927 become an impressive branch of genetics. The vast literature that has developed in this field includes some of the most precise data in biology. For the moment we avail ourselves of this information for but one purpose; namely, to assert that we have no doubt that irradiation induces gene mutations in man. This and the additional assumption that these mutations enter the gene pool of the human population are made without discussion. Arguments supporting these assumptions are to be found elsewhere (MULLER, 1950; STURTEVANT, 1955). The acceptance of these assumptions leads to the admission that our population is confronted with a radiation problem. Our discussion of this problem will differ from most; the sections that follow will review briefly concepts of population genetics; the concluding section will deal with the bearing these concepts have on the probable effects of artificially induced mutations.

INDIVIDUAL VARIATION

"Modern taxonomy is outstanding in its recognition of variation within populations. Individuals are considered not as mere replications of one another but as members of a population exhibiting a spectrum of real differences. The type-specimen was once regarded as the exemplar of a given species; individuals differing sufficiently from the type were classified as separate species. Today the type-specimen has lost much of its biological importance. Well-studied groups are represented by samples of many individuals from which estimates of both means and variances can be obtained. The type-specimen has been relegated to an administrative role; it is simply the physical counterpart of the species name.

"An appreciation of variability is essential to an understanding of change within populations. A proper approach to the problems of evolution was impossible under the view that each individual was genetically identical to every other individual of its kind. Many who fail to comprehend the extent and nature of this variability are mystified today by the development of DDT-resistance by insect populations. The same confusion is found, too, in connection with the development of resistance to antibiotics by bacteria.

GENETIC VARIATION

"The change in taxonomic philosophy was based on the development of the science of population genetics. An important corollary of MENDEL's laws is the equilibrium (HARDY-WEINBERG equilibrium) that exists between individuals of different genotypes within a population. If the genes *A* and *a* have frequencies p and q respectively, the frequencies of *AA*, *Aa* and *aa* individuals in a large, panmictic population are p^2, $2pq$, and q^2. The proportion (q^2) of individuals in a population exhibiting a rare recessive trait is always much smaller than the frequency (q) of the recessive gene itself. Many 'normal' individuals carry recessive genes in the heterozygous condition. Since the number of loci at which mutations are known is extremely large in well-studied groups, it is apparent that virtually every individual must carry at least one recessive mutation. This reasoning underlies the claim that, aside from identical twins, no two individuals are genetically alike. This reasoning, too, brought about the modification of taxonomic thought.

"In a sense, however, the geneticist retained the 'type-specimen' philosophy of the taxonomist. The type of the geneticist was the 'wild-type' gene, the allele symbolized above by *A*. The normal individual, according to this view, carried at least one wild-type allele at every locus; the non-existent ideal individual would be homozygous for these alleles at all loci. The wild-type allele was considered virtually constant for a species; the allele present in every normal individual was identical to that in all others. However, in the late 1930's even this concept of constancy was weakened by the identification of wild-type iso-alleles, alleles distinguishable only through the use of special genetic tests. The existence of differences of this order increased once more the amount of demonstrable genetic variation in populations (see, for instance, STERN & SCHAEFFER, 1943).

"The revelation by geneticists of the existence of this genetic variation in populations represented a tremendous advance for population genetics. The dominance of wild-type alleles allowed recessive mutations to accumulate in a population without imposing an undue strain upon its fitness. The necessity for possessing this concealed store of recessives was the need for adapting to novel demands imposed by a changing environment. Rates of spontaneous mutation were assumed to be established by natural selection at a level which endowed the species with an optimal amount of genetic variability. At any given time, however, the necessity for keeping these mutant genes resulted in a burden on the population.

BALANCED POLYMORPHISM

"The concept of genetic variability in the form of concealed recessive mutations failed to explain certain instances of polymorphism in nature. Polymorphism is the existence of several phenotypically distinct types of individuals in one population; balanced polymorphism is the existence of these types in fairly constant proportions over a long period of time. FISHER (1930) proposed very early that the stability of a balanced polymorphic system based on a single gene pair is assured if heterozygous individuals are favoured by natural selection over both homozygotes. Mutant alleles in cases of balanced polymorphism are tolerated not because they are concealed; they are actively selected for within populations through the adaptive superiority of heterozygous individuals.

"Our knowledge of the extent and importance of balanced polymorphism was increased by the discovery of cryptic polymorphism—polymorphism for chromosome structure—in *Drosophila*. Cytological examination of giant salivary chromosomes of *Drosophila* larvae of numerous species revealed a wealth of naturally occurring inversions. WRIGHT & DOBZHANSKY (1946) demonstrated that in artificial populations of *D. pseudo-obscura*, individuals carrying chromosomes of two different arrangements were favoured by 'natural' selection over individuals homozygous for either one of the two inversion types. DOBZHANSKY & LEVENE (1948) were able to show later that evidence for the selective superiority of heterozygous individuals was present in data that had previously been misinterpreted (DOBZHANSKY & EPLING, 1944).

"At the present time it is most profitable to assume that selection is responsible for all cases of balanced polymorphism in which the rarer gene has a considerable frequency. This view has been strengthened in human populations by evidence that heterozygosis for the gene causing sickle-cell anaemia confers resistance to malaria (ALLISON, 1954) and that various blood group genes are associated differentially with a number of human ailments (see SHEPPARD, 1955; ALLISON, 1955).

COADAPTATION AND GENETIC HOMEOSTASIS

"The early studies on inversions in populations of *D. pseudo-obscura* showed that these inversions constituted cryptic, balanced polymorphic systems within these populations. Later studies (DOBZHANSKY, 1950) indicated that a stable equilibrium was obtained in experimental populations only if chromosomes of the two arrangements came initially from the same geographical region. If they came from different localities, equilibria were not always established and, furthermore, erratic and unpredictable changes in relative frequencies occurred in different experimental populations (DOBZHANSKY & PAVLOVSKY, 1953). Chromosomes of different arrangements were said to be co-adapted by natural selection within local populations.

"The concept of co-adaptation has been extended to include cytologically invisible as well as visible factors. If selection favours individuals heterozygous at many gene loci and if combinations of alleles at each of these loci interact with those at other loci, natural selection must co-adapt the elements of any local population into an integrated gene pool.

"The concept of integrated gene pools based upon selection of heterozygous individuals of superior adaptive value is scarcely more than a working hypothesis. As a hypothesis it has proved to be a rewarding one. In our own work with irradiated populations of *D. melanogaster* the hypothesis was suggested initially by the non-correspondence between frequencies of obviously deleterious chromosomes in populations and the viabilities of individuals carrying random combinations of these same chromosomes. The explanation seemed most likely to lie in the indifference of populations to properties exhibited by chromosomes when homozygous; within populations these chromosomes occurred only in random, heterozygous pairs (WALLACE & KING, 1951, 1952). The hypothesis was tested further by an analysis of variation arising through gene recombination. Selection for homozygosis should curtail the variety of novel gene combinations that can be formed by recombination of chromosomes from the same population. Crossing-over between chromosomes from different populations can produce novel combinations even if selection in each population favours homozygous individuals; any combination of chromosomes from the two populations should yield, however, the same array of cross-over products. Experimental data obtained through an analysis of 100 cross-over chromosomes obtained from each of the 55 combinations of ten chromosome—five from each of two experimental populations—supported the hypothesis that selection favours heterozygotes (WALLACE *et al.*, 1953).

"Evidence for the integration of local gene pools has also been obtained from natural populations. WALLACE (1953, 1954) interpreted the geographical distribution of gene arrangements of *D. pseudo-obscura* as evidence for the existence of integrated gene complexes. Combinations of inversion-types incapable of maintaining the integrity of gene combinations are seldom found within the same population. VETUKHIV (1953, 1954) studied the survival under semi-starvation conditions of inter-population F_1 and F_2 hybrids and of intra-population, non-hybrid individuals. He found that a higher percentage of F_1 hybrids than of non-hybrids survive; F_2 hybrids, however, have a much lower survival rate than either of the other two classes. This has been demonstrated for three species: *D. pseudo-obscura*, *D. willistoni*, and *D. paulistorum*. The results of these experiments have been paralleled recently by KING (1955) in an analysis of DDT resistance in experimental populations. In this study the F_2 hybrids of two resistant populations of *D. melanogaster* possess less resistance than do either of the two original populations or the F_1 hybrids. Finally, recent experiments in our own laboratory (WALLACE, 1955) using *D. melanogaster* have indicated that only the inter-population F_1 hybrids have higher viabilities than the non-hybrid members of local populations. By studying three-way and double-cross hybrids it was possible to show that flies genetically as heterozygous as the F_1 hybrids frequently have much lower survival rates. These studies demonstrate the integration of the gene pools of local populations. They also demonstrate the importance of heterozygosity for selected gene complexes; this demonstration supports the earlier conclusion based on the geographical distributions of gene arrangements.

"Evidence indicating why selection might favour heterozygous individuals has also been obtained. WALLACE & MADDEN (1953) and DOBZHANSKY &

SPASSKY (1953) have re-examined the problem of the frequency of subvitals (chromosomes with slightly deleterious effects on viability) in populations. In the course of these analyses it was necessary to determine the various components of the total variance observed between tests of different chromosomes: (i) the component due to the number of flies counted in making the tests, (ii) the component representing differences between replicated cultures, and (iii) the 'true' variance between different chromosomes from a population. As reported by DOBZHANSKY & WALLACE (1953), replicated tests of homozygous individuals possessed a large between-culture variance while similar replicated tests of heterozygous individuals did not. Heterozygous individuals appear to be better buffered against the effects of these different environments. Selection may decrease total phenotypic variance by increasing genotypic variance.

"It is necessary to mention in this connection the work of LERNER (1954). Working with entirely different material and using a different approach, LERNER has reached a series of conclusions regarding homeostasis and population structure that virtually coincide with those presented above.

MUTATIONS IN NATURAL POPULATIONS

"One's concept of the genetic structure of populations determines one's concept of the role mutations play in these populations. The presence of genetic variation was originally unsuspected. Next, the HARDY-WEINBERG equilibrium and its modifications allowed accurate analyses to be made of the relation between mutation rates and frequencies of recessive mutations in populations. These mutations were regarded as a burden tolerated by natural selection because they enabled the population to adapt to fluctuations of the environment. Finally, we have a model that implies that mutations are retained in populations largely by virtue of their adaptive values in heterozygous individuals.

"The fate of a recent mutation under this model might be as follows: provided that it is not lost by chance from the population immediately, the new mutation may exist in a small number of individuals. This is a rare gene so it exists only in heterozygous individuals. These individuals carry background gene combinations as well as the mutation under discussion and so they will suffer different fates. In certain instances their destinies will not depend upon the new mutation. Among others, however, there will be some individuals favoured by selection because of the overall genotypic characteristics determined by interactions between the mutation and alleles at other loci; similarly, there will be other individuals eliminated by selection because of corresponding interactions. Consequently, the mutation in question may be preserved and, to the extent that gene recombination allows, so are the background combinations in which it was favourable. This is the course of natural selection at a low gene-frequency level. The proportion of new mutations that possess selective advantages in the heterozygous condition is unimportant: those that have an advantage, regardless of their initial proportions, will be preserved and will become part of the gene pool of the population. Similarly, the behaviour of a favoured mutation when it is homozygous is also unimportant; the selection described is based upon properties of heterozygous individuals.

The duration of the selective superiority of a given mutation does not determine its selection at a given moment; the genetic background is composed of a continuously shifting complex of mutant alleles each of which participates in a selective process like the one described.

"Our 'load of mutations' (MULLER, 1950) becomes, then, a complex burden consisting of a spectrum of mutations. At one end are those whose favourable action in any genotypic background is highly unlikely; these mutations are maintained in the population at low frequencies simply by mutation pressure. At the other end are mutations—perhaps grotesquely aberrant when homozygous—that are retained in populations through their advantageous behaviour in heterozygous individuals. Perhaps the bulk of all mutations includes those alleles that are favourable in some genetic combinations and unfavourable in others; depending upon the course of future selection these may have their position shifted towards either end of the spectrum.

"Our understanding of the problems confronting populations under this model is still inadequate. We can see that co-adaptation in the case of large blocks of genes leads to exceptionally stable examples of balanced polymorphism; this was demonstrated in the case of chromosomal rearrangements in *D. pseudo-obscura*. In looking beyond present populations by means of the geographical distribution of the gene arrangements, we can discern faintly just how stable these balanced heterozygous systems are; not only do some of them appear to have antedated species but it also appears that in some instances speciation has occurred during the preservation of these balanced systems (WALLACE, 1954). In attempting to understand some of the present systems, we lack the insight necessary to evaluate the price that different populations can pay for their genetic structures. Populations of *D. tropicalis* inhabiting Central America, for instance, possess a balanced-lethal inversion complex; for some unknown reason the populations of this single area find it advantageous to preserve this system even though half of the zygotes formed each generation are lost (DOBZHANSKY, oral communication).

RADIATION DAMAGE

"In the introduction two assumptions were made which led to the conclusion that human populations are confronted with a radiation problem. This problem arises not only from the utilization of atomic energy but also from the widespread use of X-radiation for medical and industrial purposes. A proper evaluation of the nature of this problem depends not only upon an understanding of radiation genetics but also upon a knowledge of population genetics. What bearing does co-adaptation within Mendelian populations have on the question of radiation damage?

"Under a system of co-adaptation, as under any other system, gene mutations whose average effect is to lower the viability or 'fitness' of individuals must be eliminated by natural selection. In human populations this elimination must be recognized in terms of human suffering and individual misery. Since the preponderance of newly induced mutations are probably deleterious in virtually all genotypes, these mutations will harm individuals and handicap their families. We cannot wantonly bestow pain and misery upon individuals of succeeding generations; there must be positive reasons for exposing persons to irradiation at any time. Unnecessary or irresponsible exposure cannot be justified by the negative arguments that radiations have no effects or that, at most, they induce just a few mutations.

"From the point of view of populations, the concept of co-adaptation indicates that genetic events move more rapidly than they do under the model of 'concealed recessives'. One of the fundamental aspects of the model presented above is the importance placed upon differential selection among heterozygous individuals. Negative selection for heterozygous individuals will rapidly eliminate the responsible genes from a population. If selection is positive, these genes are quickly co-adapted to the rest of the gene pool. Since the incorporation of 'favourable' mutations occurs regardless of the rarity of such mutations, we might expect that some of the radiation-induced mutations will be taken up by the genetic fabric of our population.

"It seems unlikely—barring wholesale exposure of populations to near lethal doses of irradiation—that any reasonable level of exposure will result in the extinction of human populations. The uncertainty in this matter lies in the definition and adherence to a 'reasonable' level of exposure; it is difficult to see how reason will prevail in the frenzy of technological advances that is surely approaching. I personally doubt whether atomic disasters will burden populations for thousands or even hundreds of generations with deleterious mutations; natural selection seems to act much too rapidly in eliminating semi-dominant, deleterious mutations. As we saw above (WALLACE & KING, 1951, 1952) the supply of genetic material passed on from generation to generation in irradiated *Drosophila* populations gives rise to remarkably normal individuals.

"In discussing radiation effects, geneticists make a useful distinction between somatic and genetic damage. Somatic damage does not affect the germ cells and, hence, is not transmitted from generation to generation. Regardless of the extent of this type of damage at any time, the next generation is spared its effects. Some types of genetic damage follow precisely the pattern of somatic damage; dominant lethals and dominant sterility factors are completely eliminated from populations the generation following the cessation of their induction. The model proposed for co-adapted gene pools, through its emphasis on semi-dominance, has the effect of blurring still more the transmission patterns of somatic and genetic effects. Perhaps by rendering the distinction between these two types of effects less clear, this model will allow geneticists to shift some of their concern to individuals of our own generation."

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3

GENETICS — AGRICULTURAL APPLICATIONS

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Abstract—The plant breeder has traditionally relied upon two sources for the heritable variation on which to base his selection programmes, namely spontaneous mutation and segregation following hybridization. Ionizing radiations speed up the supply of new mutations; and though most of these are deleterious (from the viewpoint of the breeder as well as the plant), a few may give agriculturally desirable characteristics. A selection programme based on irradiated material will therefore usually proceed more rapidly than one without. The main contribution of nuclear physics to agricultural genetics is that it has made available new sources of ionizing radiations. Eight of the papers presented at Geneva described investigations into the genetic action of various types of ionizing radiation on plant material under various environmental conditions. Four of them are considered in detail in this chapter, namely "The Production of Beneficial New Hereditary Traits by Means of Ionizing Radiation" by L. Ehrenberg, I. Granhall & A. Gustafsson; "Resistance to Rust Induced by Ionizing Radiations in Wheat and Oats" by W. M. Myers, E. R. Ausemus, F. K. S. Koo & K. J. Hsu; "Ionizing Radiations as a Tool for Plant Breeders" by R. S. Caldecott; and "The Effects on Plants of Chronic Exposure to Gamma Radiation from Radio-cobalt" by A. H. Sparrow & J. E. Gunckel.

It is almost a matter of definition that mutant genes are disadvantageous to a wild organism. The organism has evolved in the presence of cosmic and terrestrial ionizing radiations; therefore, it is argued, any mutational step which could occur under the action of radiations has almost certainly occurred at least once in the past, and any mutant which conferred a selective advantage would already have arisen and been incorporated into the wild-type genome under the action of natural selection. The wild-type therefore supposedly represents the optimum genome, and any mutation induced in it would represent a shift away from the optimum. In other words, it would be disadvantageous.

This argument rests on several assumptions, three of which may, at the best, represent no more than a rough approximation to the truth. First, the assumption that there exists such a thing as an ideal or wild-type genome; this is tantamount to an assertion that a given allele is always advantageous, or always disadvantageous, whatever the rest of the genome. Second, the assumption that a given allele is always advantageous, or always disadvantageous, whatever the environment. Third, that the organism is living under a regime in which natural selection is allowed to play its full role. Certainly the last two of these assumptions, and probably all three, are invalid when domesticated plants are under consideration. These live in a man-made environment, artificially enriched with plant foods, and from which competing organisms are as far as possible eliminated; and they are the subject of careful selection by man for various characteristics to suit his own needs.

Mutations may therefore occur in a domesticated plant which, though probably disadvantageous to the plant if it were living in a wild population under the full rigour of natural selection, are advantageous when considered from the special viewpoint of agricultural man. (Such mutations have been described as *positive* by GUSTAFSSON, to differentiate them from true advantageous mutations, i.e. those which are advantageous to the organism in which they arise.) Ionizing radiations are therefore of potential value to the plant breeder, in so far as they speed up the natural processes of mutation and thereby increase the supply of positive mutations. Traditionally, the plant breeder has drawn upon two sources for the genetic variability without which there can be no successful selection: spontaneous mutation and segregation following hybridization. Ionizing radiations now add a third, and chemical mutagens a fourth. The majority of induced mutations, like the majority of those occurring spontaneously, are disadvantageous, both to the plant and to man. Heavy artificial selection must therefore be exercised in any plant improvement programme based on induced mutation. But this is not a serious drawback to the plant breeder, since heavy selection is his normal procedure; discarding nine hundred and ninety-nine plants is considered a small price if the thousandth represents a material and reproducible improvement in some characteristic of economic importance.

The main contribution of nuclear physics to plant breeding has been through the greatly increased availability of sources of ionizing radiations, and especially of densely ionizing radiations, over the last few years. Before 1935 the only ionizing radiations available to biologists were X-rays, radium γ-rays and α-particles from radon; few ventured beyond the use of X-rays. However, following the introduction of particle accelerators and nuclear reactors, there have become available high-voltage electrons, β-particles, protons, fast and thermal neutrons and multicurie γ-radiation sources. This has led to a great growth of research in plant radiation genetics, ranging from fundamental research into the modes of action of the various types of radiation to applied research aimed at the production of strains of plants improved in some particular respect; much of it has been done in Scandinavia and the U.S.A. Eight of the papers presented at Geneva were in this field, and they ranged over its whole extent. Inevitably, the same or similar aspects of the subject were covered by different authors, and therefore no attempt will be made in this review to discuss all the papers in detail; instead, four representative papers, between them covering the greater part of the field, will be reproduced in part or entirety.

* * *

In their paper entitled "The Production of Beneficial New Hereditary Traits by Means of Ionizing Radiation", L. EHRENBERG, I. GRANHALL & A. GUSTAFSSON survey the Swedish work on agricultural crops and fruit trees. After briefly reviewing the history of their subject, they describe the present position and foreshadow future developments.

"Principles for the induction of positive mutations have now been worked out in a large number of agricultural species. The Swedish group treating the subject has concentrated chiefly on barley, which is a diploid self-fertilizing organism in which the mutations are easily identified, and on wheat, which is a polyploid but still self-fertilizing organism suitable for mutation analysis. In barley, in striking contrast to wheat, there is a wide range of morphological drasticity, from small scarcely perceptible changes to most profound alterations in type, even breaking the morphological frame of the variety or species. In spite of their greatly altered appearance, many mutants are surprisingly productive. This is for instance the case with the so-called *erectoid* mutants in barley, about 75 of which are now being studied. The erectoid mutation takes place, as far as we know, by changes at 16 to 20 different gene loci. Many of these mutants display features valuable from an agricultural point of view. The grain (and straw) production approaches or equals that of the mother strain; in some cases the mutant is even superior by a few per cent. At the same time erectoid mutants distinctly surpass the mother as regards stiffness of straw. Some are earlier, possess higher protein content, etc. In addition, the erectoids are able, more so than the mother line, to utilize very high nitrogen dressings, a desirable property in farming today. Other mutants, on the contrary, e.g. the so-called *bright-greens*, thrive better in a meagre soil. On the other hand, they appear to be more resistant towards certain fungi than the respective mother strains.

"The changed response to nitrogen dressing is one example of an altered reaction norm, involving the sudden appearance of new ecological requirements by induced mutation. A new *ecotype* or race has arisen. As previously mentioned, ionizing radiations induce frequent chromosomal rearrangements (which are sometimes beneficial when homozygous and detrimental when heterozygous, in contrast to lethals), leading to deviating chromosome configurations detectable in cell division. Now and then the erectoid mutations originate simultaneously with such chromosome rearrangements. This causes the formation of more or less pronounced sterility barriers in the crosses between mutants and mother strain. In fact, by one stroke and simultaneously, we may induce all the essential characters distinguishing species in nature: (i) the origin of a sterility barrier, (ii) a drastic change in morphology and anatomy, (iii) an altered ecological response, and (iv) a new karyotype detectable microscopically (GUSTAFSSON, 1954).

"It has been definitely shown, in barley and other experimental plants, that induced mutation can increase the yielding capacity of a variety, or leave this capacity intact and improve upon special characters of importance in agriculture. Besides stiffness of straw and the response to an increased nitrogen dressing, this is true also of protein or oil content, baking quality, malting properties, fibre strength and grain size, and not only in cereals but also in peas, lupines, flax, mustard, tomatoes, etc.

"A few examples of interest will be given. In Sweden, where the yellow sweet lupine (*Lupinus luteus*) is cultivated north of its centre of adaptation, it has been considered especially important to breed for increased earliness. This was found to be difficult with the traditional methods of breeding (hybridization and selection). After X-radiation of seeds a greater variation in this property was obtained, and selection for earliness became successful, without any significant decrease of the yielding capacity (TEDIN, 1954). A few X-ray varieties have already been released to the market: the *Primex* white mustard of Svalöf (ANDERSSON & OLSSON, 1954), which by an increase of grain yield as well as oil content surpasses the mother strain in oil yield per area by about 7%; the *Strålärt* (Ray Pea) of Weibullsholm, and the *Schäfers Universal* beans (*Phaseolus*) (cf. KNAPP, 1950). Numerous promising mutants in barley, wheat, peas and lupine are now being tested in large-scale experimentation; some are in fact included in the official Swedish state trials.

"The majority of highly productive mutants, especially of species represented by very high-bred and specialized varieties, such as barley and wheat, are not suitable for direct marketing. The basic use of induced mutations therefore consists of the building up of new variability, similar to that occurring in the old-time varieties of the species, but based on the highest-yielding modern varieties. The subsequent crossing of these mutants with one another or with other varieties will contribute to the gradual improvement of cultivated plants and may effect this with a considerable saving of time.

"In fruit-trees spontaneous somatic mutations (bud sports), which can be propagated vegetatively, have played a great role in the production of new market varieties. Out of 143 apple varieties of known parentage marketed in the U.S.A. and Canada during the last thirty years, no less than a quarter originated as bud sports (GRANHALL, 1954); and in pears, peaches, plums, and cherries about 10% have originated in this manner. At the Balsgård Fruit Breeding Institute, Sweden, techniques have therefore been worked out for treating scions or seedlings with different ionizing radiations, in order to induce bud sports. The investigations carried out have provided valuable information, and mutations have been obtained affecting colour and shape of fruit as well as ripening time (GRANHALL, 1954).

"In barley, the effects of different ionizing radiations are being compared extensively, partly as a first step in investigating how to direct and control the mutation process, i.e. to influence the distribution of mutation types in a special direction. These studies have so far revealed that when seeds are irradiated the total mutation frequency increases more rapidly with linear energy transfer (ion density) than does the lethalizing action of the radiation (EHRENBERG & NYBOM, 1954). The lethalizing action of fast neutrons—produced by (d, n) reactions in cyclotrons (ion density 400–800 ion pairs per micron) or in the pile centre (1 000 to 3 000 ion pairs per micron)—is about 20 times that of 175 kV X-rays (about 100 ion pairs per micron) or Co^{60} γ-rays (8 ion pairs per micron). Since, however, the mutagenic efficiency is about 40 times greater, about twice as many mutations are obtained at the 50% survival point.

"As to the distribution of various mutation types it has been shown (EHRENBERG & NYBOM, 1954) that lethal mutants, deficient in chlorophyll and other plastid borne pigments, are relatively more common after neutron irradiation, and that consequently the viable mutants, i.e. those of practical interest, are commoner after irradiation at a low ion density: twice as many mutants, relatively, are viable after X-ray treatment as after neutron treatment. With a corresponding survival of the irradiated generation the proportion of mutants of practical interest will therefore be about the same after treatment with X-rays and γ-rays on one hand and fast neutrons on the other.

"Although the material is still rather scant, it can be stated with a high degree of probability that, within the group of viable mutants in barley, the relative frequency of *erectoid* mutations increases with increasing linear energy transfer of the radiation, the frequency in the 1953 experiments being about 25% after irradiation with X-rays, 50% for cyclotron-produced neutrons, and nearly 100% after irradiation in the pile. Data from the 1954 experiments have given further evidence of this difference in relative efficiency.

"Within the group of chlorophyll-deficient mutations several lethal types can be recognized (GUSTAFSSON, 1940), viz. a colourless *albina*, a light green or yellow green *viridis* and a yellow *xantha*, which are the commonest, and in addition several rare types. Each group consists of mutants at a great many loci. The relative frequencies of these types are seemingly identical for X-rays with 100 ion pairs per micron or neutrons with 400 to 800 ion pairs per micron. When ion density is varied within a wider range it becomes evident, however, that the relative frequency of *rare chlorophyll mutations*, treated as a group, increases with increasing ion density (EHRENBERG & ANDERSSON, 1954; EHRENBERG & SAELAND, 1954).

"In addition it has been shown that variation of the irradiation conditions, other than ion density, may provoke displacements of the types of chlorophyll deficient mutations. The *viridis* type, which occurs in combination with high sterility of the irradiated generation (GUSTAFSSON, 1940), is thus more common under conditions favouring high sterility (EHRENBERG, 1955): about 22% *viridis* are obtained when moist seeds are irradiated with low doses ($\leqslant$ 5 000 r), whereas the frequency amounts to about 62% when dry seed are irradiated with high doses. The latter very high frequency approaches what is obtained when chlorophyll mutations are induced by means of the chemicals mustard gas and nitrogen mustard (MACKEY, 1954).

"Summing up, we may state:

"(i) The induction of mutations by means of ionizing radiations has given, and can give, hereditary changes of high production capacity in agricultural plants. If the mutants, although highly productive, are not suitable for direct marketing, they may form a source of variation valuable for continued breeding along traditional lines.

"(ii) It has been proved, in principle, that the spectrum of mutation types can be intentionally displaced in different directions. This is achieved, for instance, by variation of the ion density, from the minimum obtained with Co^{60} γ-rays or P^{32} β-rays, via X-rays and neutrons of different origin, to densely ionizing α-rays; and also by variation of the irradiation conditions or by the application of mutagenically active chemicals. In this way also the origination of positive, directly valuable mutations can be controlled. These results form a first step in our attempts to learn how to influence selectively the mutation of individual genes.

"Most agricultural species, even high-bred ones like barley, wheat or corn, are still rather old-fashioned as to morphology, anatomy and karyology, in spite of fifty years or more of intense breeding work. They need to be reconstructed to conform with the requirements of modern agriculture, including a high degree of mechanization and intense fertilization of the soil, thereby increasing yield and quality but decreasing labour and cost of cultivation. Recent studies by WETTSTEIN (1954, and unpublished) indicate that the structure of the straw of wheat and barley ought to be fundamentally re-patterned,

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with regard to internode number and internode length as well as cross-section area of the nodes and internodes, before the varieties can be considered fully adapted to high nitrogen dressings and intensive mechanization. In respect of internode structure barley should be changed towards wheat; in respect of cross-section area wheat, in its turn, should be remodelled so as to resemble barley. These changes can be effected by induced mutation, possibly more easily so than by hybridization of different varieties and subsequent selection. Similarly, in corn the mutual development of vegetative and generative parts ought perhaps to be readjusted in such a way as to decrease the vegetative system and increase, relatively, grain production, without unnecessary waste of nutrients and photosynthetic materials. Finally, the karyotype of some species, like barley, seems to be rather old-fashioned, remaining on an ancient status. The modernization of the karyotype may render possible a better coordination of genes and mutations in the breeding programme of the future."

* * *

An American programme aimed at the induction of specific genetic changes is described in the paper, "Resistance to Rust Induced by Ionizing Radiations in Wheat and Oats", by W. M. MYERS, E. R. AUSEMUS, F. K. S. KOO and H. J. HSU.

"Breeding for resistance to stem rust in wheat and oats caused by *Puccinia graminis tritici* Eriks. & Henn. and *P. graminis avenae* Eriks. & Henn., and for resistance to crown rust in oats caused by *Puccinia coronata* Eriks., has occupied an important place in the programme of varietal improvement of these two major field crops. Resistant varieties developed in these programmes have contributed in a large way to increased yield and stability of production.

"Extensive variability in pathogenicity within the disease organisms and great variation in prevalence of the different pathogenic races in different years have made necessary continuing programmes of searching for new sources of resistance and of incorporating the new genes into improved varieties.

"The problem is of such magnitude and complexity that the plant breeder and plant pathologist must use every technique available to them in seeking its solution. The work of GUSTAFSSON and his associates in Sweden suggested the possibility of inducing favourable mutations by irradiation. KONZAK & FREY, working independently in the United States, demonstrated that resistance to stem rust in oats could be induced by ionizing irradiation (SINGLETON, 1955). To investigate the potentialities of irradiation induced mutations in the programme of breeding for disease resistance, studies were initiated at the University of Minnesota in 1953. This paper reports progress to date in these investigations.

MATERIALS AND METHODS

"Lee, an outstanding variety of spring bread wheat, and Ajax and Clintafe, two widely adapted spring oat varieties, were used in the studies. Lee has resistance, derived from Hope and Timstein, to most physiological races of stem rust. It has been resistant in the field to all prevalent races prior to the outbreak of race 15B, to which it is susceptible.

"Ajax has the *Richland* gene which provides resistance to race 7 and several other races of stem rust. It is susceptible to race 8. Clintafe has the *White Russian* gene and is resistant to several stem rust races, including 8, but is susceptible to race 7. The *Richland* and *White Russian* genes are allelic or pseudo-allelic (KOO, MOORE, MYERS & ROBERTS, 1955). Ajax is susceptible to several prevalent races of crown rust.

"Dry seed lots of each variety were irradiated at the Brookhaven National Laboratory in 1953. Different seed lots of Lee were treated with X-ray dosages of 12 000 r and 16 000 r and with thermal neutrons at a dose of approximately $9{\cdot}94 \times 10^{12}/\text{cm}^2$. Seed of Ajax and Clintafe was exposed to thermal neutrons at the same dose used for Lee.

"Plants from the treated seed (X_1 and N_1 generation) were grown in the field at St. Paul, Minnesota. Plants from untreated seed of each variety were grown as controls. Individual panicles were harvested from each treated plant and from the controls.

"The X_2 and N_2 generations and controls of Lee were grown and tested (i) in Mexico, under a natural rust epidemic in which race 15B was an important component, (ii) in the rust nursery at St Paul, with an artificially induced epidemic in which all available prevalent races of stem rust were used, including 15B, and (iii) in the greenhouse at 78° F, using a mixture of isolates of race 15B. The N_3, X_3 and X_4 progenies of resistant N_2 and X_2 plants were grown in the field under an artificially induced epidemic, and in the greenhouse where they were inoculated with race 15B in the seedling and adult plant stages.

"Panicle progenies of the N_2 and controls of Ajax and Clintafe were grown and tested for rust reaction in the greenhouse. Progenies of Ajax were tested in the seedling stage with stem rust race 8 and resistant plants were subsequently inoculated with race 7. Progenies of Clintafe were first inoculated with race 7 and resistant plants were then tested to race 8. Resistant plants were grown to maturity and N_3 progenies of these plants were grown in the field where a natural epidemic of stem rust, caused by races 7 and 8, occurred.

"Additional N_2 and control progenies of Ajax and Clintafe were grown in the field nursery where an artificial epidemic was created using races 7 and 8 of stem rust and several races of crown rust including 202, 205 and 213. Seed was collected from the resistant N_2, N_3 and control plants for greenhouse progeny tests that are still in progress.

EXPERIMENTAL RESULTS

"The results in X_2 and N_2 generations of Lee are shown in Table 1. Results from field and greenhouse tests are combined. Since Lee is resistant in the field to prevalent stem rust races except 15B, and since 15B was an important component in the field epidemics and was used in the greenhouse tests, this combining of results appears to be justified. Two panicle progenies from X-ray treatment at 16 000 r and one progeny each from X-ray at 12 000 r and thermal neutron treatments contained segregates resistant to stem rust. All other progenies from treated seed and all progenies from the control were susceptible.

"Because of the relatively small number of control plants used and low frequency of progenies with resistant segregates, it cannot be concluded that the resistant types were the result of changes induced by the irradiation. Nevertheless, several things are strongly suggestive of that conclusion. Not only was no resistant plant found in any of the progenies from the control plants, but neither have plants resistant to race 15B been found in large populations of Lee used in breeding and genetic studies at the Minnesota station.

Table 1—Frequency of X_2 and N_2 Progenies of Lee Bread Wheat containing Segregates Resistant to Stem Rust

Treatment	Number of panicle progenies		Per cent progenies with resistant plants
	tested	containing resistant plants	
X-ray 12 000 r	1446	1	0·1
X-ray 16 000 r	530	2	0·4
Thermal neutrons	244	1	0·4
Control	250	0	0

In each case in which resistant plants were found, sister progenies from heads of the same X_1 or N_1 plant were uniformly susceptible, eliminating the possibility of heterozygosity of the treated seeds. In the segregating progenies there were one to five resistant seedlings out of an average of about 15, a larger number than could readily be accounted for by natural cross-pollination. Finally, the resistant plants and their progenies appear identical to the controls in head type, plant height, and other agronomic characters except maturity. They have been slightly later in maturity than normal Lee under rust epidemic conditions, a difference that may be attributable to effects of rust on the susceptible plants.

"In most cases N_3, X_3 and X_4 progenies of the resistant N_2 plants have been resistant to rust, indicating that they were homozygous for resistance. The segregates obtained from the thermal neutron treatment have been highly resistant both in the seedling and at the adult plant stage. Those obtained from X-ray treated material have been moderately resistant. It seems unlikely, on the basis of results reported by CALDECOTT *et al.*, that this difference in degree of resistance has any significance in relation to kind of irradiation used.

"The results in the N_2 generation of Ajax and Clintafe are presented in Tables 2 and 3. In Ajax 6 of the 988 N_2 progenies (0·6%) contained plants resistant to both races 7 and 8 of stem rust, while none was found in the controls.

"In Clintafe 2·3% of the N_2 progenies had plants resistant to both races, while 1·4% of the untreated progenies likewise had resistant plants. It is apparent that changes similar to those found in treated material are also occurring in untreated Clintafe. Clinton, the recurrent parent of Clintafe, is known to be inherently variable in disease resistance and in morphological characters, due in part at least to heritable changes that occur in the tiller primordia (MOREY, 1949).

"Field observations of Clintafe indicate that similar somatic mutations are common in it. It is noteworthy that the frequency of progenies with resistant plants was higher in N_2 than in untreated Clintafe and that the difference is of about the same magnitude as the frequency of such progenies in N_2 Ajax. Among the 558 N_2 progenies of Ajax grown in the field, 0·7% contained plants resistant to crown rust.

Table 2—Frequency of N_2 and Control Progenies of Ajax and Clintafe Oats containing Plants Resistant to Stem Rust Races 8 *and* 7 *Respectively*

Variety	Treatment	Number of panicle progenies		Per cent progenies with resistant plants
		tested	containing resistant plants	
Ajax	Thermal neutrons	988	6	0·6
	control	410	0	0
Clintafe	Thermal neutrons	434	10	2·3
	control	204	3	1·4

Table 3—Frequency of N_2 Progenies of Ajax Oats containing Plants Resistant to Crown Rust in the Field

Treatment	Number of panicle progenies		Per cent progenies with resistant plants
	tested	containing resistant plants	
Thermal neutrons	558	4	0·7
Control	149	0	0

"Resistant N_2 plants obtained in greenhouse studies were grown to maturity and N_3 progenies from them studied in the field in 1954. The number of seeds from each plant and hence the number of plants in each N_3 progeny was too small to provide critical evidence. Some progenies appeared to breed true for resistance to both races of stem rust, others bred true for resistance to one race and segregated for resistance to the other. Final conclusions regarding the behaviour of this material must await N_4 tests now in progress.

"As was the case in studies of Lee wheat, the number of control progenies and the frequency of progenies with resistant segregates are too small to warrant the positive conclusion that the resistant segregates resulted from irradiation induced mutation. Nevertheless, the results are suggestive.

"In the case of each panicle progeny containing resistant plants, sister progenies from panicles of the same N_1 plant were uniformly susceptible, indicating that the treated seeds were not heterozygous. The possibility of natural cross pollination cannot be eliminated with available data but some of these data suggest that it is not a likely explanation. The control and N_1 populations of each variety were isolated by at least 26 feet from any other oats, thus reducing the likelihood of natural crossing. Crosses between varieties with the *Richland* and *White Russian* genes are resistant to both races 7 and 8 of stem rust, as were the resistant segregates obtained in this investigation. However, the progenies of F_1 plants from such a cross segregate for resistance to both races, whereas the data available indicate that some plants obtained in this study produced offspring all of which were resistant to one race while being resistant or segregating to the other race.

"Among the N_2 progenies of Ajax and Clintafe, a number of chlorophyll deficient mutant types were found, while none was observed in the progenies of the controls. In contrast no chlorophyll deficient plant was found in any of the N of X progenies of Lee. These results suggest a difference in response of oats and wheat to irradiation insofar as changes in the chlorophyll formation mechanism is concerned.

DISCUSSION

"The results obtained with respect to induction of resistance to specific races of stem and crown rust in normally susceptible varieties, although not conclusive, are suggestive and encouraging. Resistance to the races used in this study is already available in existing varieties of wheat and oats and can, therefore, be incorporated into improved varieties by conventional plant breeding methods. Whether the same or different loci are responsible for resistance in the treated material and in existing varieties cannot be determined with present information. If the same loci are involved, the mutations obtained in the treated material in this study, even though induced by the irradiation as suggested, may not represent a major advance from the standpoint of plant breeding. If, on the other hand, new loci are involved and new sources of resistance can be induced by irradiation, the importance to plant breeding is obvious.

"Even if new loci are not involved the potentiality of induced mutation for resistance to specific races may be an important tool in some cases. For example, Lee wheat is an outstanding variety in all respects except its susceptibility to race 15B. If the resistant segregates derived from the irradiated material have not been altered in any other character than resistance to stem rust, they will be a real improvement over Lee, accomplished with less work and in fewer years than would have been possible with usual plant breeding methods and with less hazard of losing some of the desirable characteristics of Lee."

* * *

R. S. CALDECOTT, in his paper, "Ionizing Radiations as a Tool for Plant Breeders", describes how modification of various components of the environment affects the action of ionizing radiations on barley seeds.

GENERAL EFFECTS OF ION DENSITY

"*Seedling Studies*

"Earlier studies have shown that when barley seeds were subjected to a dose of X-rays sufficient to reduce seedling growth materially, the seeds were not uniformly injured and the distribution of seedling heights about the mean was skewed. It was of considerable interest, therefore, when we found that seedlings from seeds treated with any dose of thermal neutrons gave a normal distribution of heights about the mean, irrespective of any reduction in average seedling height resulting from the treatment (Caldecott *et al.*, 1954).

"All efforts to explain the lack of uniformity with X-rays, on the basis of non-uniformity either of the geometrical distribution of the X-ray beam and/or of the seed, failed. It was decided that this lack of uniformity must result from the physical nature of the radiation. From this inference, and from the fact that through capture in the barley seed thermal neutrons indirectly have a much higher specific ionization than X-rays, it seemed reasonable to assume that the difference may result from the spatial distribution of ion pairs in the irradiated material. In order to check this reasoning, and to preclude the possibility that the observed phenomena resulted from some experimental idiosyncrasy, it was decided that the results should be compared with two different sources of radiation which had specific ionizations comparable to X-rays and thermal neutrons. 2-MeV electrons and fast neutrons were selected for this purpose.

"Dormant seeds were subjected to X-rays, 2-MeV electrons, thermal neutrons and fast neutrons and then analysed to determine the distribution of seedling heights from a number of doses. To elucidate the difference between the two types of radiations (those with a high as compared to a low specific ionization), doses of each radiation which caused a comparable average inhibition of seedling growth were graphed (Fig. 1). This figure lucidly illustrates the similarity between the curves obtained with thermal and fast neutrons on the one hand and between X-rays and 2-MeV electrons on the other. It seemed logical to conclude from these data that the differences observed were related

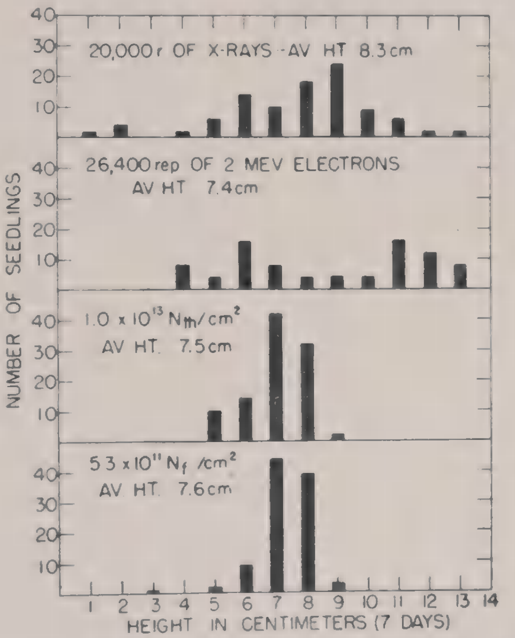

Fig. 1. Frequency distribution of seedling heights from a dose of X-rays, 2-MeV electrons, thermal neutrons and fast neutrons that reduce the average height to a comparable degree.

to the physical characteristics of the radiations and, probably, to their specific ionization (MACKEY, 1951, 1952).

"*Cytogenetic Studies*

"In one study which compared the effects of X-rays and thermal neutrons, treated barley seeds were planted in the field and permitted to produce mature plants. Cytogenetic specimens were taken at the pollen mother-cell stage and the frequency of chromosomal interchanges as a function of dose was determined. In addition, counts of plant survival were made for all treatments before the plants were harvested for seedling mutation studies (CALDECOTT *et al.*, 1954).

"By plotting the interchange frequency as a function of survival it was clearly demonstrated that more genetic interchanges could be obtained per unit survival with thermal neutrons than with X-rays (Fig. 2). The same was later shown to be true for seedling mutations. Preliminary studies conducted in the greenhouse indicated that differences of the same order of magnitude were obtained when seeds treated with γ-rays from Co^{60} were compared with seeds treated with fast neutrons (MACKEY, 1951, 1952).

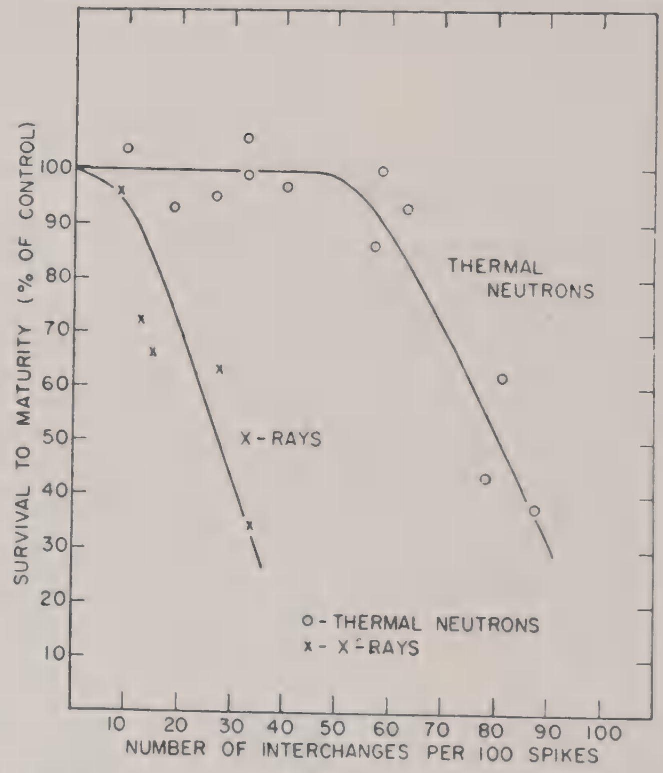

Fig. 2. Relation of survival to interchange frequency in seeds of barley treated with X-rays and thermal neutrons.

"Detailed comparisons of the kinds and relative frequencies of mutations induced with X-rays and thermal neutrons have failed to demonstrate any major differences (Table 4), even though a higher frequency of any given type of change per unit survival is obtained with thermal neutrons than with X-rays.

The same was shown to be true for the types and frequencies of interchanges. This indicates that densely ionizing radiations should be of more value to plant breeders than sparsely ionizing radiations because they give him a better probability of inducing the genetic changes which he seeks.

Table 4—Frequencies of Types of Seedling Mutants Induced in Dormant Seeds of Barley Irradiated with X-rays and Thermal Neutrons

Mutant types	Treatment				Total	
	X-rays		Thermal neutrons			
	No.	%	No.	%	No.	%
White	387	56·0	779	56·8	1166	56·5
Yellow	102	14·8	140	10·2	242	11·7
Yellow green	114	16·5	237	17·3	351	17·0
Virescent	10	1·4	42	3·1	52	2·5
Striped White and Yellow	17	2·5	36	2·6	53	2·6
Banded Shrivel	20	2·9	52	3·8	72	3·5
Others	41	5·9	86	6·3	127	6·2
Total	691		1372		1063	

INFLUENCE OF HYDRATION

"Through a combination of storing seeds in atmospheres with different vapour pressures and actually steeping them in water, a wide range of embryo water contents was obtained. By subjecting seeds with different water content levels to different types of ionizing radiations it was then possible to demonstrate differences in sensitivity modification. These differences are of particular significance to the plant breeder because they give him an indication of the variables that must be controlled when seeds are irradiated if he is to be assured of a mature plant population to study.

"(i) *Effect of Vapour Pressure*

"*Seedling studies*—Dormant barley seeds were stored over solutions with different vapour pressures (Table 5) until they reached weight equilibrium. At this time the water contents of the embryo ranged from about 4% to approximately 16% of their dry weight. Seeds representing each water content level were subjected to one of a range of doses of X-rays and then germinated and grown for 7 days in petri dishes, after which time seedling height measurements were made (Fig. 3). The data demonstrate that the sensitivity of the seeds decreased as the water content of the embryo increased from 4% to about 8%. At this upper point a plateau was reached and apparently added increments of water between about 8% and about 16% gave little further modification of radiosensitivity. Efforts to obtain similar results with both fast and thermal neutrons were essentially negative, although in some experiments there was some indication of a very slight sensitivity modification (unpublished).

Table 5—The Relation of the Water Content of Embryos and Endosperms of Barley Seeds to the Water Content of the Atmosphere in which they are Stored

Treatment	Water content of air	Approx. no. seeds	Water content of embryos and endosperms at equilibrium			
Stored in desiccator at 20° C over	(mg/litre)		Embryo		Endosperm	
			Wt(g)	% H_2O	Wt(g)	% H_2O
Dry P_2O_5	$0{\cdot}02 \times 10^{-3}$	440	0·8430	4·0	24·9801	5·0
Dry $CaCl_2$	0·20	440	0·8274	5·0	25·3545	6·0
Sat. $CaCl_2$	5·52	440	0·8813	6·0	25·7708	8·0
Sat. $NaHSO_4$	8·89	440	0·9347	8·0	26·7485	11·0
Sat. $NaClO_3$	12·82	440	0·9102	11·0	27·7838	14·0
Sat. $NH_4H_2PO_4$	15·92	440	1·0146	16·0	30·1678	19·0

"These studies point to the fact that less rigid control over seed storage conditions would be required to predict a given response when seeds are subjected to neutrons than when they are treated with X-rays. This is of particular consequence to plant breeders because they often experience difficulty in predicting biologically effective X-ray doses from year to year, and a readily available neutron source for treating their material would largely alleviate this difficulty.

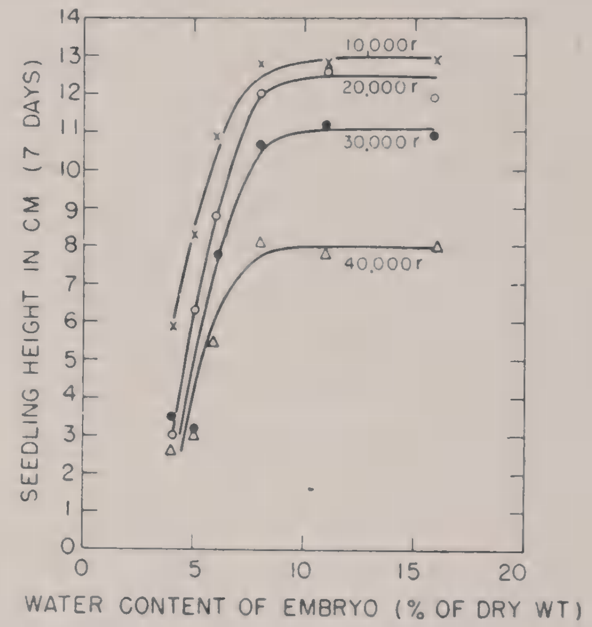

Fig. 3. Relation between the water content of the embryos of dormant barley seeds and their sensitivity to different doses of X-rays.

"*Cytogenetic studies*—A sample of the seeds having the embryo water contents listed in Table 5 were subjected to 20 000 r of X-rays and then sacrificed for cytological analyses by removing the shoot tip of the germinating seed during the first cycle of cell divisions.

"Cells at anaphase from embryos of all water-content levels were scored to determine the frequencies of normal cells and those that had chromosomal bridges (Fig. 4). As the water content of the embryo increased from about 4% to about 8%, the frequency of detectable aberrancies decreased. At the upper level of hydration, a plateau was reached which apparently was not subject to modification by further addition of water to the embryo up to 16%. A comparison of Figs. 3 and 4 indicates that there is a good correlation between the degree of seedling injury, as measured by plant height, and the frequency of chromosomal aberrations in shoot tip cells.

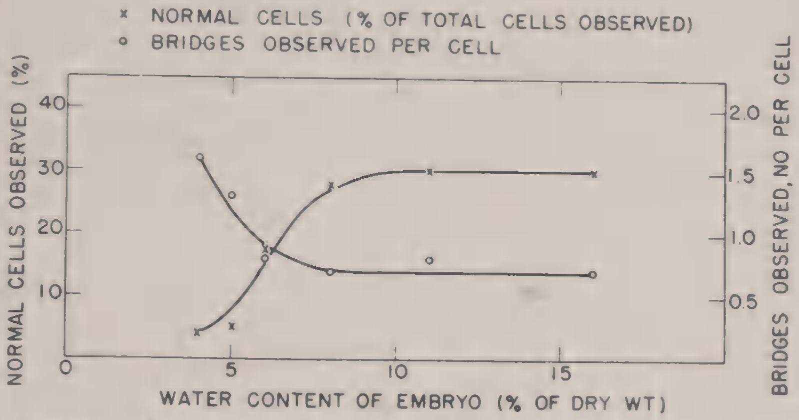

Fig. 4. The relation between the water content of the embryos of dormant barley seeds and their sensitivity to 20 000 r of X-rays as determined by cytogenetic analyses.

"These data mean to the plant breeder that he can increase the X-ray sensitivity of the genetic material of dormant seeds simply by desiccating them over phosphorus pentoxide prior to irradiation. This would be of greatest value where X-ray facilities were limited or expensive.

"(ii) *Effect of Soaking*

"Two of the factors known to limit the germination and growth of seeds are water and temperature. Water provides the working medium for the biochemical reactions incident to growth. The temperature at which growth takes place determines the rate of these reactions. If both the water content and the growth of seeds modify their radiosensitivity, it should be possible to increase or decrease the efficiency, per unit energy absorbed, of any given radiation in producing physiological and genetic changes.

"In this connection, seeds originally desiccated over phosphorus pentoxide were soaked at 22° C and 0° C for various periods of time prior to X-radiation. Following irradiation the seeds were germinated and grown in petri dishes for 7 days, at which time seedling height measurements were made. These studies showed that after one hour steeping at 22° C the seeds were much less sensitive to X-rays than controls that had been stored over phosphorus pentoxide and not steeped. However, steeping the seeds at this temperature in excess of

2 hours resulted in a striking increase in their X-ray sensitivity, so that after 6 hours they were more sensitive than dry controls. On the other hand, when soaked at 0° C the seeds just reached maximum resistance after about 4 hours' steeping and they remained at this level of tolerance for at least 8 hours before they underwent a gradual increase in sensitivity. After 24 hours at 0° C the seeds were about as sensitive to X-rays as the dry controls (Fig. 5).

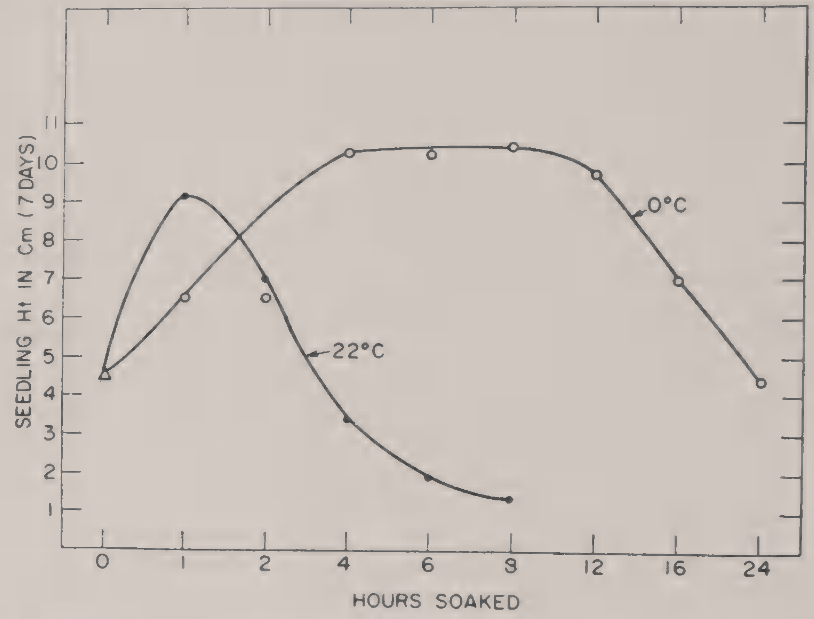

Fig. 5. Effects of soaking barley at 22° C and 0° C on their sensitivity to 15 000 r of X-rays.

"Further studies on the nature of the sensitivity changes that accompany soaking at 22° C have demonstrated that seeds soaked for about 8 hours are about 10 times as sensitive to X-rays as dry controls that have been stored over phosphorus pentoxide prior to irradiation. This increased sensitivity resulting from soaking for 8 hours is reflected by both a reduction in seedling growth rate and an increase in the frequency of chromosomal aberrations (unpublished). These studies indicate that soaking seeds at about 20° C for 8 hours or more gives the maximum increase in sensitivity that can be obtained with seeds under the conditions we have tested.

"Studies on the effect of increasing the water content of seeds by steeping prior to subjecting them to thermal neutrons have shown that the treatment reduces their sensitivity for about 16 hours at 22° C and for at least 24 hours at 0° C. Other cytogenetic studies on seeds soaked at 22° C suggest that the period at which the increased sensitivity occurs with thermal neutrons is associated with the time at which chromosome doubling takes place in cells of the seed (unpublished).

"The reduction in sensitivity to thermal neutrons that accompanies the initial stages of soaking seems to be best explained by the fact that in the unsoaked seed over 90% of the ionization results from protons and α-particles from capture of thermal neutrons by nitrogen and boron respectively. Thus, when the water content is increased, the ratio of nitrogen and boron to the total elemental constituents of the seeds is decreased. From this it follows that relatively fewer thermal neutrons would be captured by nitrogen and boron and thus the biological efficiency per neutron would decrease.

"In contrast to the observations with thermal neutrons, when seeds are soaked at either 0° C or 22° C and then subjected to fast neutrons their sensitivity increases with increased time of steeping. The rate of increase is greater at the higher temperature (unpublished). Undoubtedly some of the sensitivity increase is associated with cellular activity, but it seems likely that a considerable fraction results from the greater abundance of hydrogen for reaction with fast neutrons.

INFLUENCE OF ATMOSPHERE

"It has been well established that a number of biological materials X-rayed in the presence of free oxygen are more sensitive than when X-rayed under anaerobic conditions (HAYDEN & SMITH, 1949). There is some indication that the magnitude of this *oxygen effect* is dependent on specific ionization. Therefore, to help clarify the role of ion density in the oxygen effect, dormant barley seeds with about 10% water in the embryo were placed in atmospheres of air, oxygen, carbon dioxide, and nitrogen for 24 hours and then irradiated with either X-rays, fast neutrons or thermal neutrons. Following treatment the seeds were germinated and height measurements were taken after 7 days (Table 6).

*Table 6—The Influence of Atmosphere during Irradiation on the Sensitivity of Barley Seeds to X-rays, Thermal Neutrons, and Fast Neutrons as Measured by Seedling Height at 7 Days**

Treatment	X-rays		Thermal neutrons		Fast neutrons	
	No. seeds	Av. ht. (cm)	No. seeds	Av. ht. (cm)	No. seeds	Av. ht. (cm)
Control (no treatment)	80	12·1	147	11·6	147	11·4
Air	40	6·7	142	3·8	147	6·5
Oxygen	40	4·0	147	3·9	147	6·5
Carbon dioxide	40	7·3	149	4·0	—	—
Nitrogen	40	7·9	146	3·9	147	6·9

*Doses of radiation used: X-rays, 20 000 r; N_{th}, 1·7 × $10^{13}/cm^2$; N_f, 6·3 × $10^{11}/cm^2$.

"The atmospheres had little or no effect on susceptibility of the seeds to either neutron source of radiation. However, the seeds treated with X-rays were distinctly affected by the atmosphere in which the irradiation was given. X-radiation in the presence of either oxygen or air reduced seedling height by about one-half or one-eighth respectively over that of the seeds treated in nitrogen and carbon dioxide.

"Preliminary cytogenetic studies suggested that the oxygen effect produced when seeds were subjected to X-rays was also manifested in an increase in the frequency of chromosomal aberrations (HAYDEN & SMITH, 1949; NILAN, 1954).

"These observations are further evidence of the manner in which radiosensitivity can be modified by environment. However, it is still too soon to say conclusively whether or not it will be possible to alter the kinds and frequencies of genetic events obtained from irradiations by modifying the conditions under which materials are exposed.

"From the plant breeder's standpoint the most significant feature of this study appears to be the fact that it presents him with a means of increasing the efficiency of the X-ray facility at his disposal."

* * *

Another American programme concerned more with elucidating the many actions of ionizing radiations on growing plants than with the induction of specific heritable changes is described in the paper entitled "The Effects on Plants of Chronic Exposure to Gamma Radiation from Radio-cobalt", by A. H. SPARROW & J. E. GUNCKEL.

"Although the use of kilocurie radiation sources in experimental botany is a relatively new development, results already obtained show that such a tool can be useful in the study of both normal and abnormal plant growth. During the past six years the authors have investigated the effects of chronic exposure to γ-radiation in over 100 species of plants representing 35 different families. Several papers dealing with various aspects of this work have already been published, as well as a number of preliminary reports. However, since the technique of chronic exposure of plants to ionizing radiation has previously been investigated by only a few workers using small radiation sources, a great deal of new information is still available concerning the cytogenetic and morphogenetic responses of plants to long-continued exposures to ionizing radiation. Since a general review of the morphogenetic abnormalities induced by ionizing radiation has already been published (GUNCKEL & SPARROW, 1954), this report will deal with a few new specific examples.

METHODS

"Radioactive cobalt in the form of metallic cylinders or hollow tubes has been used consistently as the source of γ-radiation. For comparative purposes, plants have been exposed frequently to acute radiation with X-rays and in a few experiments β-radiation from P^{32} and fast electrons (800 kVP) from a resonant transformer electron beam generator were also utilized. The Co^{60} sources were used in both outdoor and indoor installations. A special greenhouse utilized 1·5 to 14 curie sources, while two different outdoor γ-radiation fields varying in size from about 2·6 to 9 acres used 16 to 1 800 curies sources. In all cases the radioactive cobalt could be remotely controlled in such a fashion that when personnel desired to enter the field or greenhouse the source could be lowered into a shield which reduced the level of radiation well below the permissible dose of 50 mr per 8-hour day. Except where special experiments required some deviation, the standard procedure was to expose plants for 20 hours out of every 24. This allows four hours each day for planting, cultivating, examination and collection of the material exposed. Although somewhat inconvenient at times, this schedule seems to be a practical one.

"The daily dose rate received by any given plant depends on the size of the radiation source (i.e. number of curies) and the distance from the source to the plant. Except for a minor correction due to air absorption, the dose rate falls off according to the inverse square law. Thus all plants growing equidistant from the source received the same daily dose rate. However, plants at any given position receive slightly less radiation per day at the end of the season than at the beginning, due to the decay of the radiocobalt. Since the decay is only about 1% per month, the change produced during the summer is of minor consequence for most of our work. In this report the daily dose rates given are those determined by measurement and calibration of the source at the beginning of the growing season each year, usually in April or May. More detailed techniques for operation of a γ-radiation field have been published elsewhere (SPARROW & SINGLETON, 1953).

TOLERANCE OF CERTAIN PLANT SPECIES TO CHRONIC GAMMA RADIATION

"Prior to the establishment of the Brookhaven γ-radiation field, there was essentially no data on the tolerance of plants to chronic γ-irradiation. During the six years since the first γ-field was put into operation the tolerance of over a hundred species of plants has been determined. Some of this information has been reported elsewhere (SPARROW & CHRISTENSEN, 1953; SPARROW & SINGLETON, 1953), but since the available information on tolerance may be of value to other institutions who wish to carry on similar work, it was decided to present a brief summary of tolerance data so far available (Table 7).

"The dose rates indicated in Table 7 indicate to the best of our knowledge the range in which severe irradiation damage (usually severe growth inhibition or dwarfing) would occur. The dose rate which causes severe growth inhibition varies greatly in the different species studied. Some species, e.g. *Tradescantia paludosa* and *Lilium longiflorum*, show severe morphogenetic effects with dose rates as low as 30 to 40 r per day, while others, such as *Gladiolus*, show little or no effect at dose rates of 5 000 to 6 000 r per day. Very few plants so far studied have shown gross morphological changes at dose rates below 15 r per day. However, chromosome aberrations and mutations have been noted in significant numbers at dose rates below 1 r per day (SPARROW & SINGLETON, 1953; SINGLETON *et al.*, 1955).

"There are undoubtedly a considerable number of factors which determine the radiosensitivity of a given species of plant. The results of our investigations seem to indicate clearly (i) that plants with very large chromosomes have a high radiosensitivity, (ii) that plants with smaller chromosomes tend to be less sensitive than those with large chromosomes, and (iii) that polyploid species within a genus tend to be less sensitive than diploid species.

"*Growth Inhibition and Stimulation*

"Previous work, with both acute and chronic exposures of ionizing radiation, has shown clearly that growth inhibition or stunting is a very common effect. Growth stimulation, however, is much more controversial, and the question has been much discussed in the literature for many years (see SAX, 1955). The interest in this phenomenon is not surprising since any treatment

Table 7—Daily Dose Rates Required to Produce Severe Radiation Effects on 79 Species of Plants

Dose rate (r/day)		
30–50	*Lilium longiflorum*	*Tradescantia paludosa*
	Taxus media	*Tradescantia ohiensis*
51–100	*Cornus florida*	*Setcreasia* sp. (4n)
	Impatiens Sultanii	*Vicia faba*
101–200	*Acer rubrum* (6n or 8n)	*Ilex* (4n)
	A. spicatum (?)	*Magnolia* sp. (?)
	Commelina coelestis (?)	*Pyrus malus*
	Cosmos	*Rhododendron* (hybrid)
201–400	*Antirrhinum majus*	*Mirabilis jalapa*
	Canna generalis	*Nicotiana Bigelovii* (4n)
	Capsicum frutescens (2n 4n)	*N. glauca*
	Chrysanthemum nipponicum	*N. glauca x Langsdorffii* (6n)
	Coleus Blumei (4n)	*N. Langsdorffii*
	Dahlia (hybrid) (8n)	*N. rustica* (4n)
	Datura stramonium	*Phytolacca decandra* (4n)
	Gossypium hirsutum (4n)	*Pisum sativum*
	Kalmia latifolia	*Prunus persica*
	Liriodendron tulipifera	*Vicia angustifolia*
	Luzula purpurea	*Vicia tenuifolia* (4n)
	Melilotus officinalis	*Zinnia elegans* (?)
401–800	*Allium cepa*	*Lycopersicon esculentum*
	Althea rosea (6n or 8n)	*Petunia hybrida*
	Celosia cristata (4n)	*Pieris japonica*
	Chenopodium album (4n)	*Ricinus communis*
	Chrysanthemum ircutianum (4n)	*Rosa* (Hybrid Tea Rose)
	Helianthus annuus	*St. Paulia*
	Ipomoea noctiflora	*Sedum aizoon* (?)
	Kalanchoë daigremontiana	*Stachyurus* sp. (?)
	Lactuca sativa	*Xanthium* sp. (4n)
801–1600	*Chrysanthemum arcticum* (8n)	*Lanophyllum pusillum* (?)
	C. lacustre (22n)	*Linum usitatissimum*
	C. yezoense (10n)	*Mollugo verticillata* (8n)
	Cucurbita (pumpkin) (4n)	*Phaseolus vulgaris* (?)
	Iris (hybrid) (4n)	*Sedum acre* (12n)
	Kalanchoë blossfeldiana	*S. album* (16n)
1601–6000	*Digitaria sanguinalis* (4n)	*Kalanchoë tubifolia* (4n)
	Gladiolus (hybrid) (6n)	*Lanophyllum texanum* (?)
	Graptopetalum Bartramii (2n)	*Luzula acuminata* (8n)
	Graptopetalum McDougallii (22n)	*L. multiflora* (4n)
		L. pallescens (4n)

Chromosome number is diploid unless otherwise stated. Question mark (?) indicates degree of polyploidy uncertain.

that can stimulate plant growth is not only of considerable scientific interest, but is also of potential economic value. We report, therefore, the following observations even though the cause for the enhanced growth is not clear.

"Plants of *Antirrhinum majus* have been grown in the γ-field over a wide dose range for four years. The first two years it was noted that toward the end of the summer certain plants growing at the higher dose rates grew much taller than average. The following year measurements of plant height were taken at dose rates varying from 14 to 600 r per day. The data indicate that at dose rates above about 125 r per day there is a gradual increase in plant height, reaching a maximum at 230 r per day. At 285 r per day the plants are still much taller than average, but at 330 r the average plant height has decreased appreciably to less than that of normal plants. Associated with this increase in plant height is an increase in average stem diameter. Increased leaf thickness has also been observed (see below).

"A number of pure species of *Nicotiana*, as well as certain interspecific hybrids, have been grown in the γ-field and the γ-greenhouse for several years. On several occasions it has been found that plants growing at a certain dose rate grew taller than plants at higher or lower dose rates and also that they bloom earlier. The exact dose rate at which this apparent stimulation occurs varies from experiment to experiment and is apparently influenced by the external or internal environment of the plant, both of which could be expected to vary considerably from experiment to experiment and from year to year.

"This example of apparent stimulation is of considerable interest, but it is not a typical response for plants in general. More commonly, the growth of plants exposed to chronic γ-irradiation is inversely proportional to the dose rate. This relationship between dosage and degree of growth inhibition is the basis for the inhibition of sprouting reported for potatoes following treatment with X- and γ-rays (SPARROW & CHRISTENSEN, 1950, 1954; SAWYER & DALLYN, 1955) and with fast electron beams (SPARROW, SCHAIRER & LAWTON, in the press), and for onions following X-radiation (DALLYN, SAWYER & SPARROW, 1955).

"*Morphological Effects*

"As indicated above, one of the most common effects of irradiation upon plant growth is growth inhibition and, more rarely, growth stimulation. In addition, the roots, stems, leaves, buds and flowers of most plants may have their normal expression altered by exposure to radiation (GUNCKEL, SPARROW, MORROW & CHRISTENSEN, 1953; SANKEWITSCH, 1952). Most of the early work, particularly X-ray effects, was reviewed by JOHNSON (1936) and later work, particularly with γ-radiation, was reviewed by GUNCKEL & SPARROW (1954). The present paper will be confined to a few unpublished examples of leaf, bud, stem and flower development following chronic exposure to γ-radiation. In all cases, the response will vary, depending upon the level and duration of the dosage and upon the species, age and physiological condition of the plants irradiated.

"*Leaves*—In most plants the leaves already present on the plant at the beginning of the exposure are changed relatively little. Rarely, these older leaves may have a restricted blade or show some growth of the blade between the veins; more commonly the leaf texture may be changed, becoming dry, stiff and coarse, or thickened and leathery.

"Young leaves formed during irradiation show a wide range of responses. In general, the leaf abnormalities include dwarfing, asymmetrical development of the leaf blade, distorted venation, a change in texture and thickening of the blade. This latter is most interesting and has been studied in some detail in *Antirrhinum majus* at 17 dosages ranging from 0·5 to 600 r per day. At the lowest dosages, the leaf was not significantly thicker than that of the controls. However, with increasing dosage there was a progressive thickening of the leaf up to at least three times that of the controls at 600 r per day. The leaves at dose rates above 240 r per day had a thick, leathery aspect, quite different from the controls. Sections were made from representative leaves and from these measurements showed that two-thirds of the increase in thickness was due to repeated cell divisions in the palisade layer. Enlargement of spongy mesophyll cells contributed about one-third to the leaf thickening.

"An additional leaf response may be reported from the 1954 γ-field studies. Plants of *Graptopetalum MacDougallii* were placed at various dosages and observed for morphological changes. Leaves which received 590 r per day during their development became not only slightly thickened but underwent considerable enlargement in length and width by comparison with controls.

"*Root development*—We have previously reported that chronic γ-irradiation of *Kalanchoë* at 1 280 r per day seems to have a stimulating effect on the production of roots from stems (GUNCKEL & SPARROW, 1954). It has been shown that acute X-radiation of localized stem areas will cause the formation of roots above the irradiated zone (CHRISTENSEN, 1954; SPARROW, unpublished). Observations on plants grown at fairly high dose rates also indicate that the root system frequently suffers severe damage. Inhibition of the formation of new roots has also been observed in irradiated sectors of the stem cuttings of *Impatiens*. In one experiment with this plant, cuttings placed in vermiculite showed good root development from the basal node of irradiated stem sectors at 2 000 r whereas sectors irradiated at 4 000, 8 000, 16 000 and 32 000 r showed no root development at all in the irradiated zone. Roots did develop, however, above the irradiated zone at all dosages and were at least as numerous, if not more numerous, at 16 000 and 32 000 r than in the lower dosages or in the controls.

"*Flowers*—With chronic γ-irradiation, flowering is generally retarded at the higher dosages and approaches that of the controls with decreasing dosages. However, in some plants, as the snapdragons and tobaccos already cited, flowering may be stimulated in a critical dosage range which varies with the species. Since most of this has been reviewed previously (JOHNSON, 1936; GUNCKEL *et al.*, 1953a, b; GUNCKEL & SPARROW, 1954) it will suffice here to mention just one further observation on *Tradescantia paludosa*. This species is of considerable interest because it has been used so frequently for experimentation and is one of the most sensitive plants so far studied. In a critical range between 13 r and 37 r per day the plants showed many abnormal axillary buds and inflorescences due principally to the induction of multiple growth centres. Inflorescences receiving 20 to 24 r per day show, after 8 weeks, proliferation by leaf-like structures and modified flowers so that by 3 weeks a globose flower head 3 to 5 cm in diameter results. Removed from the γ-field and brought into the greenhouse, these globose flower heads commonly generate a large number of vegetative shoots. At the higher dosages in this range (e.g. 34 r per day) radiation favours vegetative rather than floral production.

Accordingly, multiple, leafy shoots are borne in each leaf axil and in internodal positions and modified leaves occur in floral positions. These observations are of interest, particularly in suggesting that one might induce proliferations where the normal stem, leaf, or flower character might be lost. Such is indeed the case and this topic is of sufficient importance to discuss independently of the morphological effects already described.

"*Tumour Induction*

"Plants of the amphidiploid hybrid *Nicotiana glauca* × *Langsdorffii* have long been known to develop spontaneous tumours on leaves, stems and roots (KEHR & SMITH, 1954). Plants of this hybrid were exposed to 20 hours per day of chronic γ-radiation at dose rates of 385, 320, 210, 105, 50, and 26 r per day. The percentage of plants showing one or more tumours of significant size was recorded after 37, 44 and 64 days. The results show that dose rates above 210 r promote more tumour development than lower rates, and that longer exposures at any given rate also promote tumour development (SPARROW & GUNCKEL, 1954). For example, after 37 days' exposure, 47% of the plants at 385 r showed tumours, whereas none showed in these at 210 r or less. In the 210 r per day plants, the percentage of plants with tumours increased from none at 37 days to 33% at 44 days and up to 80% at 64 days. This increase in number of plants with tumours was paralleled by an increase in the average amount of tumour per plant. The tumour weight is greater at the higher dose rates, reaching a high of 47 and 23% of the wet weight for tops and roots respectively at 385 r per day, compared with 0·1 and 1·0% wet weight for controls. Similar, but less extensive, work with acute X-irradiation and internal β-irradiation from P^{32} indicates a similar response.

"Morphologically, at least 6 types of induced tumour growth patterns may be described. Histologically, at least two growth patterns are evident. First, a tumour may be a composite of a large number of induced growth centres, each consisting of an apical meristem, derived from the surface and subsurface layers, and a number of associated leaves. Such leafy tumours are characteristically found in axillary positions. Secondly, tumours may increase by subepidermal cell divisions and by cell enlargement, the surface layers remaining fairly discrete and nearly glabrous. After growing for some months in sterile culture, each of the tumour types may still be recognized, indicating that the induced morphological changes are not due merely to temporary physiological disturbances induced by the radiation. The specific cause of tumour induction is not known, but work is under way to determine the chemical constituents and the nutrient requirements of each of these morphological types of tumours.

PEACEFUL APPLICATIONS OF ATOMIC ENERGY IN PLANT SCIENCE AND AGRICULTURE

"The preceding sections of this paper have been devoted to brief descriptions of some of the results that we have obtained from irradiating plants with chronic γ-rays from a Co^{60} source, with fast electron beams, or with acute X-rays. It might be well to mention some of the problems raised by these investigations and to note briefly some of the prospective practical applications of ionizing radiation in plant science and agriculture.

"*Applications in Agriculture*

"By using the techniques described for growing plants in the γ-field one may obtain a significant increase in mutation rates, in some cases (e.g. *Antirrhinum*) at dose rates below 1 r per day. The potentialities of the application of ionizing radiation in agriculture and plant breeding programmes have been evaluated (TOLBERT & PEARSON, 1952; SAX, 1955; and SINGLETON, 1955) and is considered elsewhere (SINGLETON *et al.*, 1955) so will not be considered here.

"Another potential use of ionizing radiation which may prove of practical value is the inhibition of sprouting of certain vegetables. SPARROW & CHRISTENSEN (1954) and SAWYER & DALLYN (1955) have reported that exposures of potato tubers to γ-ray dosages in the 10 000 to 20 000 r range is effective in the inhibition of sprouting. Certain other undesirable changes which normally accompany sprouting are also reduced in the irradiated material. It has also been shown (DALLYN, SAWYER & SPARROW, 1955) that sprouting in sweet Spanish onions can be effectively controlled by 4 000 r of γ-rays. There is every reason to believe that the same technique will prove useful in the inhibition of sprouting of other vegetables. With the advent of megacurie radiation sources it seems not improbable that certain vegetable crops may be irradiated on a commercial scale within the next few years (BROWNELL *et al.*, 1954; KUHL & MANOWITZ, in the press). If the process is commercially adopted, the economic value of the method would be considerable. In addition to the improved storage quality, recent work from this laboratory indicates that an X-ray dose of approximately 20 000 r may be sufficient to prevent reproduction of the Golden Nematode of potato (FASSULIOTIS & SPARROW, unpublished). If this work is confirmed, and perhaps extended to other plant diseases, the application of ionizing radiation in plant disease control might be considerable.

"The difficulty in rooting cuttings of certain plants, even after chemical pre-treatment, makes it desirable to investigate further the commercial possibilities of using radiation for the induction of adventitious roots. The method might prove to be a useful and practical one.

"*Applications to Basic Problems in the Plant Sciences*

"On theoretical and practical grounds the work reported on *Tradescantia* has intriguing possibilities. The results suggest that in critical dosage ranges one may selectively discriminate between a dosage level which favours a vegetative response and another which favours a floral response (GUNCKEL *et al.*, 1953a). The mechanisms of vegetative and floral development are little understood and might be effectively studied by such methods.

"During the life of the plant various internal systems of cells are more easily modified by radiation than others (HENSHAW & FRANCIS, 1935). Further, it has been shown that radiosensitive systems may be modified by changes in the internal or external plant environment (CALDECOTT & SMITH, 1952; COOKE, 1953; MIKAELSEN, 1954). A study of these possible mechanisms of effect may help to solve some of the physiological problems of growth and differentiation.

"The development of irradiated leaves is interesting in that it shows that the rather characteristic thickening may be due to cell division or to cell enlargement. It is suggested that irradiated leaves might be good material for studying the relative roles and the mechanics of cell division and cell enlargement in the growth process, and for trying to elucidate the manner in which radiation initiates abnormal proliferation.

"The work on tumours deserves special comment (SPARROW & GUNCKEL, 1954). We feel that our studies may throw some light on the regenerative capacity of cells. More information on the growth of normal cells is necessary before too much can be said about abnormal cell growth. However, the studies under way on the chemical constituents of these tumour cells may enable us not only to grow these tumour cells more effectively in sterile culture but also to alter experimentally the growth pattern of these tumours. By additives to the nutrient medium we hope to determine the effectiveness of various compounds against radiation damage or, conversely, to induce, by additives, radiation effects in non-irradiated plants.

"A recent review of the literature on plant growth following irradiation reports many tentative conclusions which may contribute toward a solution of some of the problems raised here (GUNCKEL & SPARROW, 1954). For example, the relationship between chromosome breakage, mitotic inhibition, and growth inhibition has been considered by several workers (e.g. GRAY & SCHOLES, 1951; SPARROW *et al.*, 1952). A number of physiological disturbances are induced in plants by ionizing radiation. Some of these disturbances, e.g. auxin metabolism, have specific morphogenetic effects. Any combination of the following factors may contribute to the morphogenetic effects described in this paper: (i) growth substance production and distribution (SKOOG, 1935; GORDON, 1954); (ii) the nutritional level of the plant (e.g. GUNCKEL *et al.*, 1949); (iii) an effect upon the mechanisms of assimilation (QUASTLER & BAER, 1950); (iv) local mobilization of nutrient materials (e.g. MITCHELL, 1940); (v) organic phosphates (MCILRATH, 1950); (vi) accumulation of free amino acids (STEINBERG *et al.*, 1949); (vii) changes in enzyme activity (ALDOUS & STEWARD, 1952; BLINKS, 1952); and (viii) inhibition of synthesis (PELC & HOWARD, 1953). With the exception of mutations, most of the irradiation effects described are probably secondary effects of physiological disturbances only quantitatively different from those which occur in unirradiated plants. Radiation may thus be a useful tool for the analysis of many problems in plant growth."

* * *

Further work in the γ-radiation field at Brookhaven National Laboratory, Long Island, is described in the paper, "The Contribution of Radiation Genetics to Crop Improvement", by SINGLETON, KONZAK, SHAPIRO & SPARROW; and a similar Norwegian programme is described in "Studies on Genetic Effects in Plants of Chronic Gamma Radiation", by MIKAELSEN. GREGORY describes, in his important paper, "The Comparative Effects of Radiation and Hybridization in Plant Breeding", experiments in which peanuts (*Arachis hypogaea*, L.) were crossed and exposed to X-rays over one or more generations, with analysis of the fruit yield variances. Finally, MURATI & MORIWAKI, in their paper, "Genetic Effects induced on Plants", describe various heritable changes in rice and other plants found growing in the neighbourhood of the bomb-damaged towns of Hiroshima and Nagasaki.

Between them these eight papers review a branch of science which had its origins in the X-ray experiments of STADLER and of MULLER more than twenty-five years ago; it now promises to achieve an economic importance comparable with that of the hybrid corn industry in the United States.

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THE USE OF RADIOACTIVE ISOTOPES IN THE STUDY OF CERTAIN AGRICULTURAL PROBLEMS

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Abstract—Radioactive isotopes have been employed in many types of agricultural investigations apart from nutritional studies which are referred to in another chapter. Wide use of isotopes, especially P^{32} and Co^{60}, has been made in studies of the migration of insects. The investigations described range from the study of the dispersal of populations of mosquitoes, locusts, blackflies, grasshoppers and pine weevils to that of the movement of individual cutworms and wireworms in soil. Automatic recording equipment has been designed for the latter purpose. Comparable methods have been used to study the dispersal of pollen. The potential value of radioactive isotopes to sterilize insects and thereby eradicate insect pests has been demonstrated. By the release of males sterilized with γ radiation from Co^{60} in numbers sufficient to compete with normal males, screw-worm flies have been eradicated from an island 170 square miles in area.

The elucidation of the mechanism of action of selective herbicides and fungicides has been greatly facilitated by labelling techniques. Isotopes afford a greater ease of detection and estimation than ordinary chemical methods. These in general are insufficiently sensitive for the estimation of these substances.

Information on root grafting and the translocation of minerals in trees has also been gained by the use of radioactive isotopes.

The boundaries of agricultural research are so broad that it would be impracticable to marshal in one chapter all investigations which come within its scope. Elsewhere in this volume studies in genetics, plant breeding, animal physiology and plant nutrition are reviewed and much reported there is of direct importance to the advancement of agricultural research. Biochemical and other fundamental biological investigations will also undoubtedly influence agricultural progress in due course. Thus a full appreciation of the application of tracer methods to agricultural research can be gained only by considering all the aspects of biology represented by the chapter headings of this volume. However, a number of important applications of tracer methods to agricultural problems fall outside this classification. They form the subject of this chapter.

STUDIES OF DISPERSAL AND BEHAVIOUR

An interesting new approach to the study of insect movement has been made possible by the use of radioactive isotopes. The earlier work in this field has been reviewed in detail by Lindquist (1952) and Dahm (1953). At the Geneva conference, Spinks (1955) enumerated the characteristics desirable in the radioactive isotopes used in such studies, namely: suitable half life, ease of application and recognition, absence of significant effects on the insect, and persistence throughout its life. These points were illustrated by reference to experiments with mosquitoes, blackflies, grasshoppers, wireworms and cutworms. Field experiments in Saskatchewan had shown that large numbers of adult mosquitoes could be labelled economically by keeping larvae in the fourth instar stage of development in a 0·1 μC per ml solution of P^{32} (in the form of orthophosphate) for 24 hours at a density of 1 larva per ml, and then allowing the larvae to complete development to the adult stage in their original or normal habitat. It was found that larvae lost 50% of the absorbed activity within 2 days of removal from the active solution, but subsequently the loss was relatively slow. The average retention of activity through to the adult stage was about 15%. Control experiments indicated that larval mortality was no greater in solutions containing 0·1 μC P^{32} per ml than in inactive media. In one experiment approximately 450 000 larvae were labelled in this way and released in a pond. In the following month about 500 000 mosquitoes were caught in the neighbourhood and of these 84 were radioactive; 19 flies, 3 parasites and 1 leaf hopper were also found to be radioactive. Labelled mosquitoes were found as far as 7 miles from the release point, but the majority were within one-eighth of a mile of the point of release.

It was necessary to modify the method used with mosquitoes for work with the blackfly. In laboratory experiments, blackfly larvae were reared in 5-litre jars containing water circulated and aerated by compressed air. It was found that from 2 to 4 larvae per ml could be kept in a solution of 0·2 μC P^{32} (as orthophosphate) per ml without excessive mortality and that an adequate amount of activity was absorbed in 24 hours. After transferring the larvae to an inactive solution, there was a relatively large loss of radioactivity in the first two days, but the loss thereafter was small. In one experiment, 300 000 larvae were tagged but of 16 000 adults caught subsequently, only one was radioactive. This poor recovery was ascribed mainly to inadequacies in collecting methods.

In a study of the dispersal of grasshoppers, 20 000 nymphs and adults were allowed to feed on wheat seedlings 6 inches high, in a cage 4 square feet in area for several hours. The wheat seedlings had been sprayed with 0·5 mc P^{32} in 50 ml solution. Approximately 14% of the activity was taken up and retained by the grasshoppers. Loss of radioactivity by the grasshoppers was high for the first few days. Thereafter losses were small, even during moulting, and survival was normal. The dispersal of nymphs in the second instar and of adults of *Camnula pellucida* (Scudd) and *Melanoplus mexicanus mexicanus* (Sauss) was studied in this way. It was found that insects released on bare cultivated fields showed no ability to orientate themselves and move toward a food supply. The average rate of movement at 70° F was approximately 7 yards per hour. Seven days after release the pattern of dispersal appeared to be the result of random movement modified by a response to wind direction.

A method of studying locust dispersals was reported by KETTLEWELL (1955). Locust hoppers or adults were allowed to feed on maize and grass grown in solutions of P^{32} or on wheat bran impregnated with a labelled solution. The author's intention in using two methods of feeding was to determine whether the rate of absorption of the labelled phosphate was affected by its chemical form. The phosphorus in the maize is described as organic, that on the bran as inorganic. No difference was observed between the treatments. This result is not surprising since a large part of the labelled phosphate in the maize plants must have also been in an inorganic form. 50% of the ingested P^{32} was rapidly lost from hoppers but further loss was negligible. Treated hoppers showed a slightly higher mortality rate and a slower rate of metamorphosis than untreated ones. Adults similarly excreted a large proportion of their activity in the first 24 hours, but some activity was retained for up to 5 weeks. At the level of P^{32} used, treated insects could be detected from a distance of 10 inches even after two weeks had elapsed from the time of treatment.

KETTLEWELL pointed out that since locust hoppers cast their skins, radioactive isotopes form the only adequate method of marking. Labelling with P^{32} could give much useful information about hopper movement and death rate. However, although this is the only available method of marking, KETTLEWELL regarded the cost of equipment and the necessity for trained staff as a considerable drawback. For studies with adult insects he concluded that tracers offered no real advantage over the colour paints which have been widely employed.

In the foregoing investigations Geiger-Muller counters were used to detect β radiation from the labelled insects. In contrast ARAGAO, FROTA PESSOA & MARGEM (1955) used nuclear emulsions to detect α-particles from the isotopes with which insects had been labelled. This technique was considered to be considerably more sensitive than that of electronic counting methods. Moreover it permitted the use in small quantities of long lived α sources such as thorium and uranium. Both prepared plates and liquid emulsions were used. For a field experiment mosquito larvae were allowed to develop in a 1/10 000 solution of thorium nitrate, the cultures being maintained for 3 months. Forty days after the cultures had been started, the collection of flies was begun at stations situated 200 m apart along 4 different directions. Groups of flies from each station were incinerated and transferred to glass slides for the preparation of autoradiographs. It was found that mosquitoes caught in the first 15 days showed no significant radioactivity, but all those caught later showed a great number of α tracks. The furthest collecting station was 800 m from the point of release, and it was therefore apparent that the maximum range of flight of the mosquitoes was at least this distance.

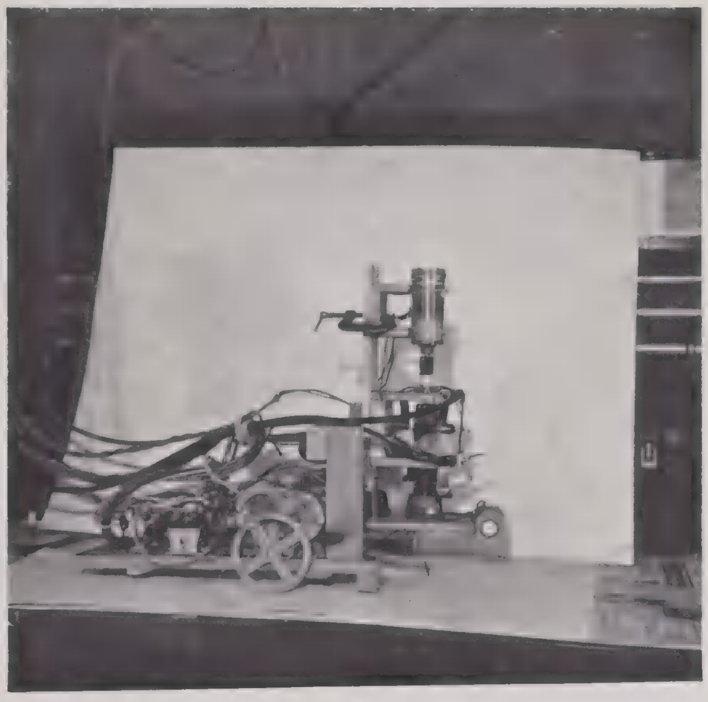
A different technique was used to label the white-pine weevil in a study reported by SPINKS (1955). Co^{60} as nitrate was dissolved in cellulose acetate and acetone and applied externally to the elytra. The tagged weevils were then released in a plantation of white pine. Of 64 individuals labelled with 0·2 to 0·5 mc each, 21 were still alive after two months. This is about the usual percentage survival for untreated specimens under similar conditions, but it was thought that a smaller amount of radioactivity might have been better since in the following spring only 19% emerged compared with 56% in a control group. The labelled insects could be detected from a distance of about 2·75 metres. Spinks also mentioned that radioactive carbon had been used to study the action of DDT on houseflies, and that radioactive arsenic had been used to label mealworm larvae and tomato hornworms.

The studies so far described were concerned with the mass labelling of insect populations. Much information can be obtained however by labelling individual specimens. SPINKS has labelled wireworms and cutworms by inserting or attaching externally minute pieces of radioactive cobalt wire (20 μC Co^{60}). In initial experiments, a Geiger-Muller counter, suitably calibrated, was used to estimate vertical and horizontal movement of the insect in the soil. Later the method was greatly elaborated by the construction of an apparatus capable of continuously following and recording the movements of the labelled insect in the soil. (Plate I.) The apparatus has been fully described by GREEN & SPINKS (1955b). It incorporates a sensitive head, rotating on a vertical axis which emits an error signal when not directly over the insect. The amplified signal is applied to a servo mechanism which causes the apparatus to move until it is once more vertically above the insect. Insects labelled with 20 μC Co^{60} could be located from a distance of 40 cm, or could be followed at a depth of 10 cm from the soil surface, with an average position error of a half centimetre. This method has been used to measure the rate of movement of the prairie grain wireworm in response to temperature, moisture and food. Wireworm larvae were found to move very quickly at temperatures above 90° F, and more slowly at lower temperatures. It was concluded that the optimum temperature for their development was between 72° and 80° F. They were observed to avoid dry soil when offered a choice of moist or dry conditions, but sometimes entered the dry region when food was placed there. The movement of larvae towards food appeared to be a random process. In other studies larvae of the red-backed cutworm were used to determine the fate and underground activity of larvae placed in open cages of the type used for chemical control studies and the observation of the habits of the larvae.

The methods of studying insect movement and behaviour by the use of isotopes are still being developed but already they are proving in many cases to be a useful new tool to the entomologist.

Radioactive isotopes have been used to study another type of dispersal problem, namely the dissemination of pollen. DEMARLY (1955), who described this application, pointed out that conventional genetic methods for studying pollen dispersal are subject to several disadvantages. When the extent of the cross pollination of plants of two different colours is utilized as an index of pollen distribution, it may happen that the vigour of plants is correlated with flower colour, or that insects may visit preferentially flowers of one particular colour. Moreover, it may be difficult to ensure that the plants used are homozygous for the character studied. In DEMARLY'S experiments *Medicago sativa* (Lucerne) was used as the test plant and P^{32} was chosen as marker since pollen grains are rich in phosphorus owing to their high nucleic acid content. Quantities up to 1 mc P^{32} were applied to the roots and were found to be readily distributed through the plant. It is reported that no radiation damage was observed. The pollen reached maximal radioactivity after 9 days; after that period it was possible to detect satisfactorily a group of 10 grains. It was found that 30% of the pollen from a labelled plant was deposited on plants in its immediate neighbourhood. Up to a distance of 4 m the amount of pollen decreased very rapidly and the maximum distance of dispersal was 10 m. These results are attributable to the very short range of flight of the most active pollinating insect (*Bombus terrestris*). An accuracy considerably greater than that obtainable by genetic means is claimed for this method of studying pollen

A



B

distribution. It was also possible to estimate the proportion of auto and allo-pollen by determining the total number of grains and the activity of the pollen on a stigmatic surface. Although the amount of auto-pollen was considerably higher than that of allo-pollen, fertilization by the latter was 40 times more frequent; it was concluded that there was a heavy selection against the auto-pollen, which appeared to result from the slower growth of the pollen tube. This work has an important practical application in determining the minimum distance of isolation for producing seed of a specific variety.

The Control of Insect Pests by γ-ray Sterilization

An ingenious and successful application of radioactive isotopes was described by BUSHLAND, KNIPLING & LINDQUIST (1955). During 1954, screw-worms, *Callitroga hominivorax* (Cqrl.), were eradicated from the island of Curacao, Netherlands Antilles, by means of a new method of biological control—insect sterilization.

The screw-worm fly is a calliphorid fly which is parasitic in the larval stage. The female deposits her eggs in a mass of about 200 on the edge of any abrasion in the skin of a warm-blooded animal. The eggs hatch in 12 to 24 hours, and the larvae feed as a colony on the living muscle tissue. Infested wounds attract other flies which oviposit, and untended animals may be literally eaten alive. Almost invariably infested animals die if not properly treated with a larvicide. The larvae complete feeding in 5–6 days and then crawl from the wound and burrow into the soil to pupate. The pupal stage lasts about 8 days in warm weather. Adult flies emerge, work their way to the surface of the soil, and crawl up on the nearest vegetation to expand their wings and fly away. They mate when 2 to 4 days old, and at the age of 6 days the female is ready to lay eggs. The adults usually die of old age in about three weeks.

In nature the screw-worm is an obligatory parasite on warm-blooded animals; if the host animal dies before the larvae have completed two-thirds of their development, they cannot survive. However, the larvae can be easily reared in the laboratory on a mixture of ground lean meat, citrated beef blood, and water with formaldehyde to retard putrefaction.

The screw-worm is tropical and semitropical in its distribution, and survives throughout the year only in those parts of the Americas where the winters are mild. In the United States it was formerly limited in its winter distribution to regions bordering on Mexico. In 1933 however, screw-worms were accidentally introduced into Georgia as a result of the shipment of infested cattle from Texas. The infestation from Georgia spread into Florida, where the insects found a winter climate suitable for their survival. During the summer months screw-worms spread over the Southeastern States doing millions of dollars of damage each year. In average winters the cold kills screw-worms north of the Florida peninsula. Thus if the overwintering population in Florida could be destroyed, screw-worms might be eliminated from the Southeastern States.

Attempts to control the flies by good husbandry and the use of larvicides failed. Attention was therefore turned to the possibility of sterilization by radiation. Since the females mate only once, the release of a sufficiently large number of sterile males would increase the proportion of infertile to fertile eggs. Thus if sterile males were released in sufficiently high numbers, the eradication of the natural population might be effected. X-rays and later γ-rays from Co^{60} were used to sterilize pupae or adult flies for this purpose. In early trials a dose of 5 000 r proved sufficient for sterilization; the only other observed effect was a slightly reduced longevity of the irradiated adult males. A dose of 7 500 r was subsequently adopted since female flies irradiated with 5 000 r were able to produce some eggs, which might have been confused with eggs from normal females. At the higher level of irradiation they produced no eggs.

In a field trial on Sanibel Island 2 miles off the Florida coast, the fertility of egg masses collected from wounded goats declined to 25% after the release of sterilized males and in 8 weeks the natural population appeared to have been eradicated. However, 4 weeks later normal flies were again active; this was presumed to be due to the nearness of the Florida coast and other islands. A second field trial was carried out on Curacao, 40 miles from the coast of Venezuela. The small size of the island (170 square miles), the high population of screw-worms, and the isolation made Curacao ideal for the experiment; 5-day-old pupae were irradiated and flown to Curacao. The flies emerged within a few hours of arrival and were distributed by aeroplane over the island in lanes one mile apart. Releases were made twice weekly, the flight lanes being shifted a half mile each time.

The activity of screw-worms and the efficiency of the release of sterilized flies were measured by examining the egg masses collected from wounded goats. Eleven goat pens, each containing 8 goats, were established at suitable sites over the island. Each week two goats in each pen were infected by making a small incision in the skin near the shoulder and implanting 100 newly-hatched larvae. When the larvae were 3 days old they were killed with benzol. The wounds were attractive to flies from the time of infestation until several days after the larvae were removed. Thus, there were always at least two wounds at each goat pen to attract ovipositing flies. Before the release of sterilized flies, native screw-worms deposited about 15 egg masses per goat pen per week and all the egg masses were fertile. After the release of sterilized flies at the rate of 100 males per square mile per week for 8 weeks, the fertility of the egg masses collected from wounded goats declined to 85 per cent. This reduction was, however, not sufficient to affect the fly population, which actually increased during a period of favourable weather.

One-half of the island was then treated with sterilized flies at the rate of approximately 400 males per square mile per week, while distribution at the rate of 100 was continued on the other half. The higher rate proved to be so effective that it was later adopted over the whole island in an effort to attain complete eradication. During the first 4 weeks of this treatment approximately 70% of the eggs collected were sterile. This high sterility caused a marked depression in the number of insects in the subsequent generation. Since the release of sterilized insects was maintained at a high rate, there was an even greater percentage of sterility in the next generation, which led to a reduction in the number of egg masses collected. Complete control was attained in approximately eight weeks. Only two egg masses were collected thereafter and both of them failed to hatch. The goat pens were maintained and the release of sterilized flies was continued for a total period of five months, but there was no further evidence of normal fly activity.

Bushland and his colleagues believe that the Curacao experiment demonstrated that it may be practical to use sterilized flies to eradicate screw-worms from the Southeastern States of U.S.A. In ordinary winters screw-worms survive in Florida over an area of approximately 50 000 square miles. An eradication campaign in Florida would involve rearing, sterilizing, and releasing about 300 times as many insects as were required for the Curacao experiment. It is thought that procedures for rearing, sterilizing, and distributing 50 million flies can be perfected so that within 2 years a campaign can be undertaken with reasonable confidence of success.

Several requirements must be fulfilled for this method to be successful. First, a low natural population must exist or the population must be reduced by some means so that an excess of sterilized males can be released. The insect must be easily reared in large numbers in the laboratory. The mating behaviour of the males must not be adversely affected by sterilization, and native females must be willing to accept sterilized males. Preferably the females should mate only once, but on theoretical grounds multiple matings should result in the production of infertile eggs in a ratio similar to that of released sterilized to native males. It is also necessary to have methods of measuring the insect population per unit area, so that the numbers required for release can be accurately estimated. Obviously the area in which eradication is attempted should be isolated or protected by quarantine or other measures against reinfestation.

Each species of insect attacked in this way will present numerous problems, and a large amount of research on the effects of irradiation, on the habits of the insect, on population trends of the species, on the rate and extent of migration, and on other problems is necessary before the feasibility of sterilization as a means of control can be determined.

HERBICIDE AND FUNGICIDE STUDIES

Crafts (1955) reported on the use of labelled compounds in research on selective herbicides. He pointed out the incorporation of C^{14} into 2, 4-D (2, 4-dichlorophenoxyacetic acid) had greatly facilitated the study of its translocation in plants since it enabled radioautographic procedures to be used. With preparations of high specific activity (8–9 mc per mol), doses of 2, 4-D could be used which caused little or no disturbance in normal plant function, while enabling good autographs to be obtained. By killing and autographing plants at different stages the process of translocation could be studied. In addition to 2, 4-D, urea, phenyl-acetic acid and benzoic acid were used and it was intended to extend the work to include 2, 4, 5-trichlorophenoxyacetic acid, 3, para-chlorophenyl, 1, 1-dimethyl urea, maleic hydrazide, and amino triazole.

The results so far obtained by Crafts and his collaborators lead them to conclude that: (1) 2, 4-D can penetrate the plant cuticle, migrate across the mesophyll, and move in the phloem in quantities that are not immediately injurious to these tissues. (2) 2 4-D is carried in the phloem and it moves only with food materials. (3) The toxicity of 2, 4-D is greatest in young growing tissues. (4) 2-4 D is broken down and lost from living plants, presumably as a result of metabolic processes.

From these generalizations Crafts drew several conclusions relating to the action of 2, 4-D. It was considered that in entering the plant 2, 4-D must pass a lipoid barrier. In some regions this consists of the cuticle, in others of the cuticularized walls of the substomatal chambers, in addition to cell membranes. The ester of 2, 4-D should be most effective, the free acid intermediate, and the salts least effective in crossing this barrier. The process may be facilitated by certain adjuvants having surfactant properties. Once through this barrier, the herbicide passes from the lipoid phase and migrates through living cells to the phloem. Here the acid is superior to the esters and the heavy esters are more mobile than the short chain aliphatic esters. Movement occurs in the phloem from regions of synthesis (mature leaves) to regions of utilization of foods (young leaves, flowers, buds, cambium, roots). Injury to the roots occurs only when the roots are growing and not simply storing food reserves. Failure to kill was considered to result from failure of the final response rather than from the failure of translocation.

CRAFTS ended his paper with this comment: "Although these final conclusions draw upon research and deductions that have not involved use of the labelled herbicide, many physiological interpretations of our results have been quickly and positively verified by tracer work. Such verification would have taken years of painstaking work if attempted by classical plant physiological methods. And the labelled materials are enabling us to plan and conduct studies that would be impossible by conventional methods. Thus they are making an outstanding contribution to the present day chemicalization of agriculture."

Other investigations using C^{14} labelled 2, 4-D have recently been reported by BLACKMAN (1955). Extensive investigations on the relationship of the effects of growth regulators on the growth and the absorption of nitrogen phosphorus and potassium by test plants have suggested that this relationship is correlated with the concentration of growth regulator or some derivative at cell level. This in turn is affected by the rate of absorption of growth regulator from the external solution. The techniques of radioisotope assay provide a convenient method of measuring the very small amounts of growth substance absorbed.

The rate of absorption of 2, 4-D by *Lemna minor* has been studied by estimating the C^{14} content of the fronds after immersion in active solutions. It has been found that there was a very rapid entry in the first few minutes followed by a steady rate of accumulation for 1–2 hours, when the rate falls off to zero. After the first few minutes of absorption the Q10 of uptake is of the order of 2·4. In higher concentrations (16–44 p.p.m.) a considerable loss of growth regulator follows. This is not due to respiration of the C^{14} but to a loss of the unchanged 2, 4-D to the external solution. Present evidence suggests that this may not be due to a purely physical process. It is noteworthy that a concentration which induces loss to the external solution after 1–2 hours may not cause a decrease in growth rate until 4 days later.

The absorption of 2, 4-D by coleoptile segments of *Avena* and *Triticum* has also been studied. In contrast with *Lemna* the rate of absorption by *Triticum* coleoptiles does not fall off appreciably in 22 hours. Absorption is accelerated by the reduction of the pH from 6 to 4 but is not directly proportional to the concentration of undissociated molecules. The increase in the length of *Avena* coleoptiles is proportional to the amount absorbed from solutions when the concentration is below 0·25 p.p.m. but at higher concentrations a linear relation no longer holds.

BLACKMAN considers that some of the differences recorded between tissues may be due to variation in the physiological level of the growth regulator rather than to variation in specific reaction of the test plants. Further important results may be expected from the study of the mechanisms of action of growth regulators by this technique, especially when material of high specific activity becomes more widely available.

The distribution of a labelled growth regulator has also been studied by Russian workers (briefly reported by KURSANOV (1955)) who employed 4-iodo-phenoxyacetic acid labelled with I^{131}. This substance was found to accumulate especially in actively growing young flowers and fruits.

MILLER & MCCALLAN (1955) described the use of radioactive isotopes in tracing fungicidal action. They pointed out that plant diseases, the majority caused by fungi, result in annual crop losses in the United States alone at almost three billion (10^9) dollars. Control measures which keep such losses from greatly exceeding this figure are aimed almost exclusively at the prevention of the germination and the subsequent growth of fungus spores. Fungus spores are very small; from 100 000 to 200 000 000, depending upon the species, weigh one milligram. Before radioactive isotopes became readily available, quantitive studies of the affinity fungus spores for toxicants could be carried out only with great difficulty, As a result almost no information was available regarding the quantity of toxicant fungus spores took up when exposed to solutions or suspensions of fungicidal agents nor was there information on the quantity required to prevent germination. Radioactive isotopes have made such measurements possible and have also been useful in studying the penetration of fungicides into host plants.

MILLER & MCCALLAN determined the rates of uptake and the quantities required to reduce germination for spores of representative species of fungi using elemental S^{35}, S^{35}-labelled ferric dimethyldithiocarbamate, C^{14}-labelled 2-heptadecyl-2-imidazoline and 2, 3-dichloro-1, 4-naphthoquinone, and Ag^{110}, Ce^{144}, and Hg^{203} (in ionic form). A 50% reduction in germination was obtained when quantities of from 85 to 10 000 micrograms were taken up per gramme of spores of different species. Unlike the other substances sulphur was not accumulated by spores but was reduced to hydrogen sulphide and released. Lethal doses were taken up from dilute solutions (1–10 p.p.m.) within one-half to five minutes. The fungicides studied were much less toxic per unit weight of spores than many animal poisons, insecticides, herbicides, or bactericides. The authors suggest that it should be possible to develop better fungicides since there is no reason to believe that fungi are more difficult to kill than other organisms.

Studies on the interaction of various toxicants with respect to their absorption and subsequent release by fungal spores were carried out to determine whether competition occurred for receptor sites. Some toxicants such as 2-heptadecyl-2-imidazoline, cerium and silver, were stated to have no receptor sites in common since the saturation of spores with one of these toxicants did not interfere with the subsequent uptake of the others. Toxicants competing for the same receptor sites would interfere with each other and their rates of uptake would be reduced when they were used simultaneously. The presence of one toxicant sometimes increased the rate of uptake of another, for example, when silver and mercury were used together, the rate of uptake of mercury was increased and that of silver was decreased. This result was considered to indicate that silver has an effect in increasing the permeability of the cell membrane. It was suggested that studies on effects of various substances in decreasing or increasing uptake should lead to more efficient practical use of fungicides and thereby lessen the losses caused by fungus diseases.

Effects on the permeability of cell membranes were studied by determining the release of phosphorus compounds into the ambient solutions when spores labelled with P^{32} were exposed to various toxicants. Silver caused the greatest release of cell contents even at doses which did not completely inhibit germination, while other ions such as mercuric, cadmium, zinc and copper could produce 100% inhibition of germination with comparatively little effect on cell permeability. Silver which had been taken up by spores exchanged rapidly and practically completely with silver subsequently added to the medium. The other toxicants did not exchange so readily and the degree of exchange was usually less than 50%. Mercury absorbed as mercuric ion exchanged to a greater extent with mercurous ion than with mercuric ion. Practically no exchange occurred with spores treated with 2, 3-dichloro-1, 4-naphthoquinone. This was taken as evidence that the naphthoquinone had undergone chemical change on contact with the spores.

In preliminary experiments with labelled ferric dimethyl-dithiocarbamate, 2-heptadecyl-2- imidazoline, and 2, 3-dichloro-1, 4-napthoquinone it was found that there is little uptake of toxicants by host plants. It was thought that radioactive tracers would prove especially useful in studying uptake by host plants and the possible activity of some substances as systemic fungicides.

STUDIES OF TRANSLOCATION AND ROOT GRAFTING IN TREES

KUNTZ & RIKER (1955) described the use of Rb^{86}, Br^{82} and I^{131} to measure the translocation of nutrients in trees and also movement between trees joined by root grafts. Branches or excavated roots were inserted directly into bottles containing the isotope, or the isotope was directly applied to chisel cuts in the trunk.

It was found that the normal rate of upward movement of the isotopes in the sap streams of oaks in full sunlight was between 1·5 and 3 feet per minute when the humidity was relatively low. Downward movement also was rapid in roots which were grafted to those of adjacent trees but there was little translocation to ungrafted roots. In northern pin oaks, the isotopes were distributed throughout the crown. Radioactivity was detected within 20 minutes in most branches, twigs, and leaves of oaks 35 feet high. In bur oaks and white oaks, the rate was comparable, but radioactivity appeared only in branches having vascular connections with narrow, vertical streaks originating at the chisel cuts. Thus, certain branches were radioactive; others were not. The rate of movement of isotopes in the sap stream varied considerably throughout a 24-hour period. When the leaves were dry, rapid movement occurred during the day from the time the sun first shone until it fell below the crown canopy. Late in the day when both light intensity and temperature decreased and relative humidity rose, the rate of movement was greatly reduced. During the night it fell to only a few per cent of the daytime value. Comparable reductions in the rate of movement were obtained when trees were covered with light-proof canvas. During daylight dense clouds reduced movement considerably and still more marked effects were obtained when the leaves were wet from dew, rain, or artificial sprays with water. No movement occurred at 0° C or below. Seasonal variations in the rate of upward movement were also examined. It was found that the average rate of movement remained uniformly high throughout the growing season until autumn coloration appeared. Thereafter the rate of movement was only a few per cent of the summer figure until the buds swelled in spring. Moreover during this dormant period, movement was almost confined to the southern side of the trees.

Observation of the movement of tracers between trees was found to be a convenient method of detecting root grafts. Observations made in this way were confirmed by excavating root systems. Some species showed very extensive grafting especially the northern pin oak, large toothed aspen and red pine. The most extensive graft system recorded connected 36 trees. In contrast white and black spruce and balsam fir showed no root grafts. Grafting of varying frequency was observed in red, bur and white oak, sugar maple, white pine, white birch, jack pine and quaking aspen. Only once in many hundred observations was a graft observed between two species. Root grafting was not confined to old trees. Isotopes moved in both directions across the graft union but the most frequent direction of movement was from the dominant to the suppressed tree. It was suggested that this effect may assist the survival of the suppressed tree.

These investigations have clarified the manner in which the oak wilt disease develops and spreads from tree to tree in local areas. Oak wilt is caused by the fungus, *Endoconidiophora fagacearum* Bretz, which invades the water-conducting vessels. Sudden wilting, premature defoliation, and death normally occur in infected northern pin oaks. In bur oaks and white oaks, which in contrast to the pin oak show few root grafts, wilting of the leaves on scattered branches is usually the only symptom and infected trees often live for several seasons. The movement not only of solutions but also of particulate material was traced with isotopes. Measurements were made of the rate and distance radioactive spores of the oak wilt fungus were carried in sap streams. A concentrated spore suspension was treated for 1 day with 1 mc of $Ag^{110}NO_3$, repeatedly washed and resuspended in 1 mc of $Na\ I^{131}$. The spores were doubly labelled with insoluble $Ag^{110}\ I^{131}$. Although their weight was increased, the radioactive spores were soon detected in the terminal branches of an oak 5 feet high. Studies of host responses to fungus invasion demonstrated that the upward movement of radioactive isotopes in the transpiration streams of infected northern pin oaks was reduced by 85% 3 to 4 days before foliage wilt appeared. A further decrease occurred during the development of moderate and severe wilt symptoms; movement in trees with severe wilt often being reduced by 99%. Similar reductions were found in the flow of water through branch sections cut from wilting trees. Microscopic examinations showed that tyloses and gums formed in the xylem vessels throughout the outer annual rings of the sapwood. They consistently preceded leaf wilt, indicating that vascular plugging had effectively blocked water conduction.

Differences in the reaction of other oak species to infection were detected by means of radioisotopes. In infected bur oaks, radioisotopes moved normally until 3 days before foliage wilt began. After wilt development, movement continued only in unobstructed portions of the outer annual ring and radioactivity was detected only in the symptomless branches. As with infected pin oaks fungus distribution, vascular plugging, and development of wilting were shown to be closely associated. The continued downward movement of isotopes into and through grafted roots of diseased oaks indicated that vascular plugging was limited in the roots. Isotopes moved through roots even after aerial portions of the tree had died.

The information on disease transmission by root grafting gained in this work has been found of value in devising control measures. According to circumstances the severing of roots or the killing of trees in zones surrounding the sources of infection is the recommended procedure. In lawn and park areas where individual trees were of great value, severing all root connections to a depth of 36 inches or more between wilting and adjacent healthy trees usually prevented further local spread. Since soon after wilt development the fungus frequently moved from infected trees into nearby trees, severing root connections between 2 or 3 rows of trees appeared advisable. In woodland areas where individual trees were of relatively low value, poisoning healthy oaks within 25 feet of the wilted trees usually confined the infection. Root kill was essential. The width of the poisoned barrier depended on species, age, stand density, and accessibility.

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THE USE OF RADIOACTIVE ISOTOPES IN THE STUDY OF PLANT NUTRITION

I. THE MECHANISM OF IONIC ACCUMULATION BY PLANTS

II. PLANT-SOIL RELATIONSHIPS

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III. THE ENTRY OF NUTRIENTS INTO PLANTS THROUGH STEM, LEAF, AND FRUIT, AS INDICATED BY THE RADIOACTIVE ISOTOPES

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Abstract—Radioactive isotopes have been widely used to study both physiological and agronomic aspects of plant nutrition. The sensitivity of tracer methods and the opportunity they provide for studying the movement of labelled fractions of substances in steady-state systems has facilitated the examination of the mechanisms whereby ions enter cells from the external medium and are subsequently transferred across the cytoplasm. Recent work on this subject is reviewed.

In the study of plant/soil relationships procedures depending on isotopic dilution and isotopic exchange have made it possible to measure labile nutrient fractions in soil. The phosphate ion has received particular attention and it is apparent that no simple relationship, applicable to widely contrasting soils, exists between labile phosphate and the quantity of phosphate which plants can absorb. Large-scale field experiments with labelled fertilizers have not in general yielded important new information except when special problems are under investigation, such as the movement of phosphate in soil and the effect of placement and the granular size of fertilizers. The physical properties of soils have also been studied.

The absorption and utilization of nutrients applied to the foliage of plants has been extensively studied. Considerable interest attaches to this question in view of the limited responses which occur under some circumstances when fertilizers are applied to the soil.

I. THE MECHANISM OF IONIC ACCUMULATION BY PLANTS

An experiment on salt absorption by plants which Hevesy carried out in 1923 was the first demonstration of the practical value of radioactive tracers in biological research. The most spectular discoveries by this technique have since lain in other fields but they have none the less been largely responsible for recent advances in the interpretation of absorption mechanisms. Among the first quantitative studies of major significance were those of Overstreet and his associates (Overstreet & Jacobsen, 1946). The usefulness of the method has lain in two directions; the sensitivity with which tracers can be detected has made it possible to study the movement of quantities of ions which were previously undetectable, and it has been possible to distinguish materials entering in a given period from those previously or subsequently absorbed. This latter characteristic has enabled the movement of a labelled fraction of an ion to be examined under steady-state conditions or where the net movement is in the opposite direction.

At Geneva, EPSTEIN & HENDRICKS (1955) reviewed the detailed experiments they have undertaken to elucidate the mechanism of active transport. The interaction of a number of pairs of ions has been examined with respect to their absorption by detached barley roots, and it has been found that linear relations obtain between the reciprocal of the quantity of nutrient absorbed in a four hour period and the reciprocal of the external concentration. In the study of enzyme kinetics it has been shown by LINEWEAVER & BURK (1934) that a similar linearity exists between the reciprocals of the velocity of an enzyme reaction and the concentration of the substrate. To interpret his results EPSTEIN has therefore found it convenient to adapt the LINEWEAVER-BURK procedure, assuming that a carrier in active transport is analogous to an enzyme, while the ion absorbed corresponds to its substrate. It is assumed that the effects of interfering ions are comparable with those of inhibitors or alternative substrates in enzyme studies. The occurrence of competitive inhibition (i.e., competition for the same postulated carrier) is therefore inferred when the interfering ion alters the slope but not the intercept of the LINEWEAVER-BURK reciprocal plot. This effect is manifest when selenate (0·1 m.e./litre) is added to a range of concentrations of sulphate (Fig. 1). If on the other hand the interfering ion alters the intercept of the curve, "non-competitive" inhibition is assumed. Conclusions reached by this method on the manner in which a large number of ions compete with Rb^+, Br^-, Sr^{++} and SO_4^{--} are shown in Table 1. Deviations from the relationships indicated in the Table have however been noted, for example when concentrations are increased, ions recorded as "non-competing" may show the characteristics associated with "competitive" inhibition. While many data appear susceptible to this type of kinetic treatment exceptions have been noted. Using carrot discs, RUSSELL & AYLAND (1955) found that the linear relationship

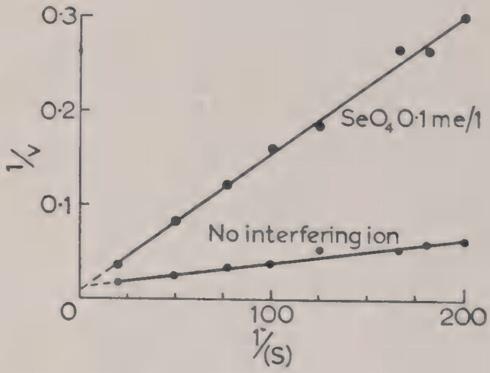

Fig. 1. Double-reciprocal plot of sulphate absorption by excised barley roots. Concentration of $K_2S^{35}O_4$, (S), in m.e./1. Rate of absorption, v, in m. μ.e./gram fresh weight/3 hours. (EPSTEIN, 1955.)

of the reciprocals of velocity and concentration did not hold for the absorption of caesium from a wide range of concentrations and they concluded that the enzyme-kinetic model did not hold for this tissue. HAGEN & HOPKINS (1955) found a similar non-linearity for the absorption of phosphate by detached barley roots but reconciled their results with the Epstein model by postulating that the H_2PO_4 and the HPO_4^{--} ions are both absorbed but by different carriers—a conclusion at variance with that of VAN DEN HONERT (1933). More critical experimental procedures and the examination of alternative kinetic models would appear to be necessary before any final interpretation is warranted.

Table 1—Competing and Non-competing Ions

(EPSTEIN & HENDRICKS, 1955)

Substrate ion	Competing ions	Non-competing ions	Reference
Rb^+ (Rb^{86})	K^+, Cs^+	Ba^+, Li^+	EPSTEIN & HAGEN (1952)
Br^- (Br^{82})	Cl^-, I^-	NO_3^-	EPSTEIN (1953a, b)
Sr^{++} (Sr^{89})	Ca^{++}, Ba^{++}	Mg^{++}	EPSTEIN & LEGETT (1954)
SO_4^{--} (S^{35})	SeO_4^{--}	$H_2PO_4^-$, NO_3^-	Unpublished data

It is now widely believed that the salient barrier to free ionic movement is at the inner as opposed to the outer surface of the cytoplasm. Thus it has been envisaged that an "outer space" or "apparent free space" exists in cytoplasm into which diffusion is possible from the external medium. Following BUTLER (1953) EPSTEIN had endeavoured to measure this volume in tracer experiments. Using the anion sulphate, he calculated values for the "outer space" exceeding 25% of the volume of the tissue—a conclusion similar to that of BUTLER. In contrast with anions he regards cations as entering by exchange. This accords with the evidence of RUSSELL & AYLAND (1955) that competition occurs between monovalent cations in absorption periods of 5 minutes with tissue slices, detached roots and intact plants. These workers concluded that exchange or absorption processes control the initial inward flux and that the quantity of ions entering tissues in an exchangeable form is no index of the volume of the tissue in which it has equilibrated.

Difference in interpretation should not however be allowed to obscure the fact that the investigations here cited, as well as many others (e.g. HOPE & STEVENS, 1952), point to the conclusion that a rapid entry of ions occurs when tissues are placed in salt solutions, that the ions can be readily lost if the solutions are changed, and that this process is distinct from the active transport mechanism. Similarly it is to be noted that, although no interpretation of the mechanism of active transport is at present universally accepted, the types of mechanism envisaged in the majority of investigations, e.g. EPSTEIN *loc. cit.*, SUTCLIFFE (1955) and RUSSELL (1955a), have the common characteristic that the existence is postulated of metabolically produced carriers which restrain both anions and cations. These interpretations contrast with the view of LUNDEGARDH (1946) that anions alone are actively transported and that the electron transfer in the cytochrome—cytochrome oxidase system is the directly operative force.

Few detailed investigations of active transport have been carried out with intact plants. Although it is obvious that the detached roots or tissue slices favoured by the majority of workers are preferable on grounds of convenience, the advent of tracer methods has markedly facilitated qualitative studies using intact systems. As RUSSELL and MARTIN (1953) have emphasised the sensitivity of the method is such that observations can be made over periods so short that weight changes are negligible and the amount of nutrient absorbed is insignificant by comparison with that already in the plant. Thus it is possible to study in plants of *equal* nutrient status the absorption and transport of ions from solutions of widely differing concentrations. Moreover in one important direction the complexity of the intact plant can simplify interpretation. In fragmentary tissue systems discrimination between ions held by exchange processes and those restrained in the active transport mechanism necessarily rests on indirect evidence such as observed isotopic exchange rates. In the intact plant, on the other hand, the appearance of ions in plant shoots can provide unequivocal evidence that they have been actively conveyed across the root. Taking advantage of this opportunity preliminary experiments with combinations of ions similar to those employed by EPSTEIN have been carried out (RUSSELL, 1955a). It has been found that the interaction of potassium with rubidium is markedly different with respect (a) to the absorption of rubidium (b) to its transference to the shoot. It is therefore possible that interpretations of ionic interactions in the active transport mechanism may not be valid if the only quantity determined is the flux of ions between the external medium and the tissue. Great importance therefore attaches to the facilities modern methods provide for studies with intact plants; not only is the interpretation of their behaviour the ultimate problem of plant physiology but also they provide a most favourable opportunity for the critical evaluation of postulated mechanisms. The final test of the value of observations made with tissue fragments may be regarded as the extent to which they assist in the design of such experiments.

II. PLANT-SOIL RELATIONSHIPS

THE wide extent to which tracer methods are being employed to study the nutrition of plants grown in soil is indicated by the fact that eight contributions at the Geneva conference were directed wholly or in part to this subject (DEAN, 1955; F.A.O., 1955; KLECHKOVSKI, 1955; KURSANOV, 1955; MITSUI, 1955; RUSSELL *et al.*, 1955; SOKOLOV, 1955; SPINKS, 1955). The material presented ranged from reviews to reports of detailed studies.

Phosphorus^{32} has been employed in the great majority of investigations; not only are many of the most urgent practical problems of plant nutrition concerned with phosphate but also the isotope is one of the most readily available and easy to handle. Calcium^{45}, sulphur^{35}, zinc^{65} and cobalt^{60} have however been widely used. DEAN (1955) in an exhaustive review, more especially of work in the United States, notes that some 300 publications have already appeared and illustrates the principal types of tracer application. Since references to all but the most recent results can be found in a number of sources (e.g. HENDRICKS & DEAN (1952), COMAR (1955), FRIED (1953), HERBST (1953), BEHRENS &

FERNANDEZ (1953)) consideration here will be confined mainly to questions on which new information was presented at the Geneva conference.

FACTORS AFFECTING THE AVAILABILITY TO PLANTS OF SOIL PHOSPHATES

The limited ability of plants to absorb phosphate—especially from many acid soils—is a major agricultural problem. Conventional chemical methods have been found inadequate to assess the ability of many soils to provide phosphate to plants or to elucidate the mechanism responsible for the inability of plants to absorb added phosphate in certain soils. This situation has been an added incentive to the use of radioactive phosphate. Two types of tracer procedure have been employed to study the reactions of phosphate in soils:

(a) The estimation of the isotopically exchangeable phosphate in soils
(b) The determination of Larsen (L) or "A" values.

Both methods give measures of the labile phosphate in soils.

Isotopically exchangeable soil phosphate was first investigated by MCAULIFFE *et al.* (1949) who showed that an initial rapid exchange reaction was followed by a slower process. Subsequent investigators have observed the same effect (WIKLANDER, 1950; RUSSELL *et al.*, 1954). In the estimation of exchangeable phosphate, complications arise from the fact that the precipitation or "sorption" of phosphate occurs simultaneously with exchange. After examining the procedures of previous workers RUSSELL *et al.* (1954) adopted the following procedure. Aliquots of soils were shaken in labelled orthophosphate solutions and after different time intervals the external solution was assayed. The isotopically exchangeable phosphate (E_t) and the sorbed phosphate (S_t) at time (t) were calculated by the equations:

$$E_t = \frac{x_t}{y_t}\, y - x \qquad\qquad S_t = x - x_t$$

when x and y were the amounts of P^{31} and P^{32} originally present in solution and x_t and y_t the values determined at time t. This estimate of E_t is valid only if the sorbed phosphate remains in isotopic equilibrium with the external solution. This condition was satisfied in the soils studied by them and in subsequent investigations (RUSSELL *et al.* 1955).

The Larsen (L) value, better known as the "A" value, was first determined by LARSEN (1952) and has since been extensively used by many workers (FRIED & DEAN, 1952; GUNNARSON & FREDRICKSON, 1953; DEAN, 1955; SPINKS, 1955). Although investigators have differed in the interpretations they have placed on their results, experimental methods have been generally similar. If labelled phosphate is intimately mixed into a soil and plants are subsequently grown in it, the amount of soil phosphate L with which the labelled phosphate has been apparently diluted on absorption by the plant can be calculated by the equation:

$$L = \frac{Y_f\,(X_p - D)}{Y_p} - X_f$$

when X_f and Y_f are the quantities of P^{31} and P^{32} added to the soil, X_p and Y_p are the total plant content and D is the phosphate content of the seed.

Experimental procedures have varied principally in two respects. Some workers have carried out determinations in the field when the intimate mixing of added and soil phosphate throughout the rooting zone is impracticable; others have conducted pot experiments with thoroughly mixed soil. Whether significant errors arise due to inadequate mixing does not appear to have been assessed with precision. The majority of workers have employed labelled phosphate accompanied by significant quantities of carrier. SOKOLOV (1955) had criticised this procedure on the grounds that the phosphate balance of the soil is upset. RUSSELL *et al.* (1955) likewise employed very low carrier levels and considered that the advantage of this procedure is that the phosphate absorbed by the plant is identical with the value obtained in untreated soil. Since however they found that *L* values were unaffected when the carrier was varied by a factor exceeding 10^3 it appears that results are not invalidated by the use of high carrier levels.

Whereas evidence has been obtained that radiation from labelled phosphate may reduce the phosphate absorption by plants it appears from the work of FOSTER (1955) that measurements of the *L* value are less subject to error from this cause. In pot experiments with tropical crops using a wide range of activities of P^{32} he found that even when the activity was such that phosphate absorption was reduced, the ratio of P^{32} to P^{31} absorbed by plants from soil *in which the tracer had been thoroughly mixed* was unaffected; thus valid *L* values could be obtained. In the light of the findings of RUSSELL *et al.* (1950) that the ratio of P^{32} to P^{31} absorbed by plants may be affected by radiation when P^{32} is *not* uniformly distributed throughout the soil, it would seem that *L* value measurements could be invalidated under such circumstances.

Whereas measurements of exchangeable phosphate (*E*) have been relatively little used in the study of the availability to plants of soil phosphates the LARSEN procedure has been widely applied in field experiments, especially in the United States of America and in Canada (DEAN, 1955; SPINKS, 1955), and the term "*A*" value has been introduced on the grounds that the value is a measure of the plant available phosphate in the soil.

RUSSELL *et al.* (1955) have described pot-culture experiments undertaken for the dual purposes of examining more critically the relationship between isotopic measurements of soil phosphate and plant uptake and of elucidating the mechanism of "phosphate fixation" in acid soils. Measurements of isotopically exchangeable phosphate *E*, phosphate sorption S, the Larsen value *L*, and uptake of phosphate by test plants were made in four soils (Table 2) which ranged from a calcareous alluvium, in which no apparent fixation of phosphate occurred (Soil I), to a basaltic acid soil (Soil IV) from which plants were unable to absorb significant quantities of phosphate in the absence of fertilizers. It is apparent from the table that phosphate extracted by 1% citric acid in 24 hours did not give a satisfactory measure of the amount of phosphorus the plants could extract. The soils were used either in their natural state or after the addition of varying quantities of KH_2PO_4. Prior to some experiments the soils were stored moist for periods up to one year after the addition of phosphate. The values of *E* reported are those found after 7 days of shaking with labelled phosphate, by which time the rate of exchange had become slow. The values of both E and L were reproducible under a wide variety of conditions. S varied with the phosphate concentration of the equilibrating solution.

Table 2—Characteristics of Soils Investigated
(RUSSELL *et al.* 1955)

Soil	I Calcareous alluvium	II Medium-heavy loam derived from silurian and old red sandstone	III Loam: basaltic	IV Loam: basaltic
Origin	Thames Valley	Edinburgh	Greenhall, Aberdeen	Northern Ireland
pH	7·6	6·0	5·8	5·5
Phosphate extracted in 1% citric acid (mg P/5g soil)	0·56	0·93	0·89	0·64
Relative values for available phosphate determined by plant absorption	1	0·8	0·2	<0·01

When aliquots of *each* soil, to which different quantities of phosphate had been added, were compared, absorption by plants increased approximately linearly with both E and L (Fig. 2). In no case did absorption amount to more than a very small fraction of the labile phosphate. This simple relationship did not however obtain for the comparison of *different* soils. Fig. 3 shows the results of an experiment with three soils, to which different levels of phosphate had been added 118 days prior to the experiment and stored either moist or dry in the intervening period. In the figure the soils are arranged from left to right in order of decreasing plant uptake. It is apparent that neither E nor L provided any guide to the relative absorption of the test plants in the different soils. Plant absorption however showed a clear relationship to the reciprocal of the values for sorption. In considering this relationship it is to be borne in mind that the soils sorbed phosphate to very varying extents in the order Soil I < II < III. Thus, when the concentration of phosphate in the solution in which soils were equilibrated was within the range in which sorption by Soil I could be precisely measured, the solutions used with Soil III were virtually depleted. This difficulty could not be overcome by employing solutions of different concentration as this would have affected the extent of sorption. None the less the procedure placed the soils in the right order with respect to sorption.

The extent of sorption of a soil may be regarded as bearing an inverse relationship to the thermodynamic activity of phosphate in the soil solution or to SCHOFIELD's phosphate potential (SCHOFIELD, 1949). Thus the results in Fig. 3 suggest a dependence of plant absorption on the phosphate potential of the soils though different methods would be necessary for any precise relationship to be determined. This relationship between plant uptake and the reciprocal of sorption did not hold in all comparisons of soils which contrast sharply in the phosphate treatments they have received. The influence of factors not yet defined is therefore envisaged.

In all soils approximately linear relations were obtained between *E* values and the quantity of phosphate with which the soils had been enriched. When the soils were examined either immediately after the addition of phosphate or following short periods of dry storage the increment in *E* equalled the amount of phosphate added in soil I but in the other soils the corresponding values were approximately 70 % of the added phosphate. Storage, especially at field capacity, reduced this value significantly. Linear relationships were also apparent between the *L* value and the quantity of phosphate added. However there were significant differences between the *L* values obtained with different test plants in the more highly sorbing soil. The uptake of phosphate by rye was compared with that of barley in some experiments (Table 3). Whereas both species showed similar results from the soil which yielded most phosphate, the ratio of the uptake by rye to that by barley increased progressively as the quantity of

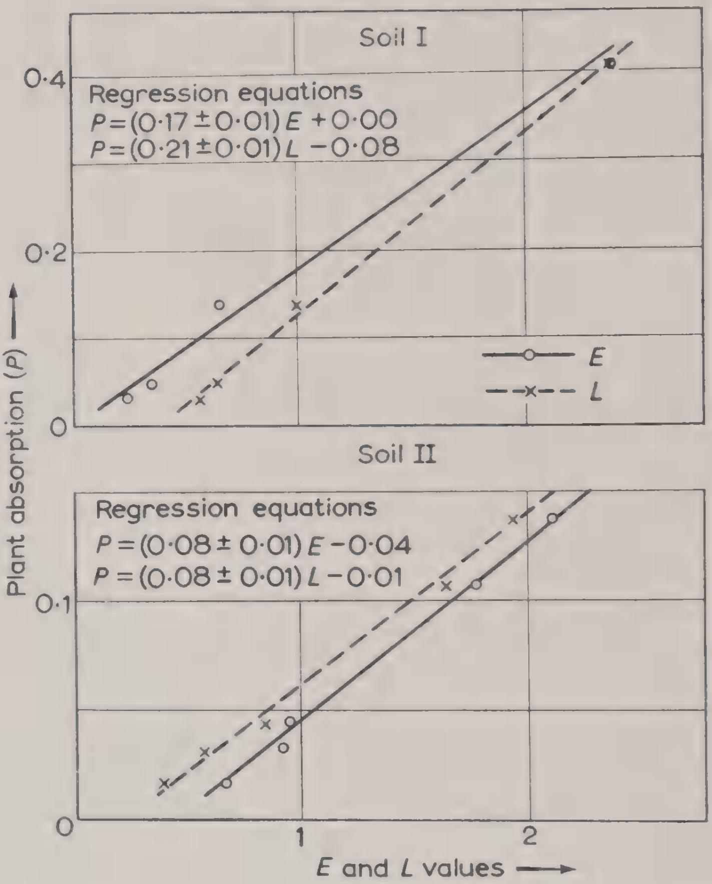

Fig. 2. Relationship between plant absorption and *E* and *L* values in Soils I and II. Results expressed as mg P/5 g soil. (RUSSELL *et al.*, 1955.)

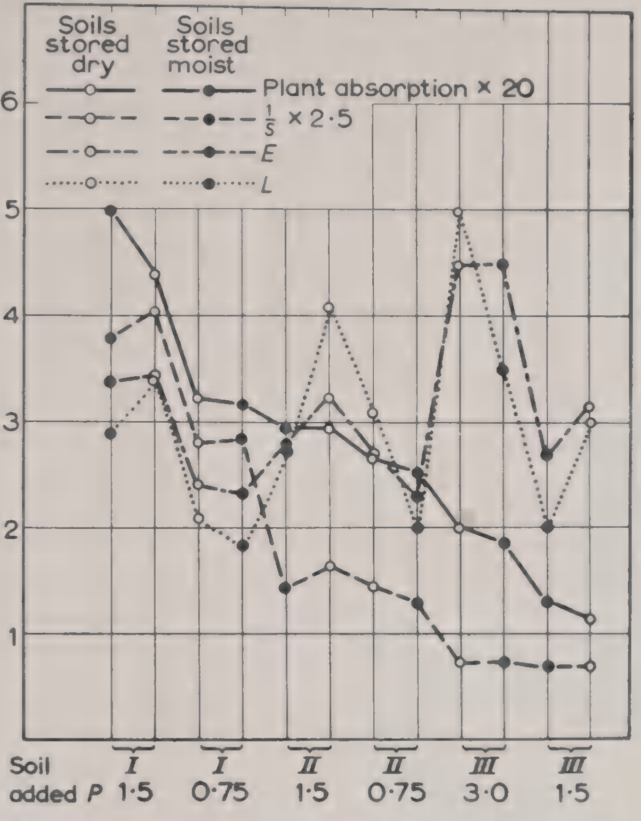

Fig. 3. Plant absorption, *E*, *L* and reciprocal of *S* determined in soils enriched with phosphate to varying extents and stored moist and dry for 118 days prior to experiment. Test plant: rye. Values expressed as mg P/5 g soil. (RUSSELL *et al.*, 1955.)

Table 3—Comparison of Absorption and L Values for Rye and Barley
(RUSSELL *et al.* 1955)

Soil	I	II	III	IV
Phosphate added (mg P/5g soil)	1·5	0·0	1·5	1·5
Uptake of phosphate (mg P/pot) 28 days after planting				
Barley	8·74	2·00	1·49	0·25
Rye	8·74	2·68	2·76	1·36
Ratio rye/barley	1·00	1·34	1·85	5·44
L value (mg P/5g soil)				
Barley	1·82	0·50	1·63	0·94
Rye	1·80	0·49	1·74	1·80
SD (5%)	0·08	0·08	0·14	0·16

N.B.—Soils III and IV without added phosphate could not be included in this comparison because of the negligible uptake of phosphate by the plants.

phosphate taken up by the barley decreased. The *L* value of the soils however was the same whether rye or barley was used as the test plant, except for Soil IV from which the uptake by both plants was very low. On this soil higher values of *L* and greater absorption of phosphate were found with rye and, in other experiments, with cabbage than with barley. Since this soil had the strongest affinity for added phosphate, this result could be interpreted as showing that rye and cabbage can take up phosphate from very dilute solutions more strongly than barley. This suggestion is supported by water culture experiments in which the absorption of phosphate by rye and barley was compared over a wide range of concentrations at pH 6. When the external concentration was low (i.e. 0·001 p p m P) the absorption of rye was markedly greater than that of barley but the reverse was true at high concentrations (e.g. 10·0 p p m P). The resultant interaction between plant species and external concentration was highly significant ($P < 0{\cdot}001$).

Consideration was also given to the effect of iron and aluminium ions in the absorption of phosphate and the authors conclude that whereas ferric ions can directly affect the absorption of phosphate the aluminium ion has no such direct effect. Reduced phosphate absorption induced by aluminium appeared to reflect the inhibition of metabolic processes. It is suggested that organically bound iron restrains the phosphate ion in a complex in which the atom ratio of iron to phosphorus is one.

These results indicate that the labile phosphate content of different soils may bear no consistent relationship to the amounts of phosphate which plants absorb; soils containing large quantities of labile phosphate from which plants are able to absorb only very small quantities have been shown to sorb large amounts of phosphate. Initially the sorbed phosphate is in a largely labile form though in the course of time it may be rendered non-exchangeable. Russell *et al.* therefore conclude that the description "available" cannot be applied to any chemically distinguishable soil phosphate fraction, and that the inability of plants to absorb phosphate in the highly sorbing soil is not initially attributable to the precipitation of phosphate in insoluble forms but rather to the lowering of the "phosphate potential" of the soil. They note also that plant physiological factors and the effect of other ions, especially iron, must be taken into account.

Regarding the use of labelled phosphate in studies of this type, they emphasize that labile phosphate measured by isotopic exchange or dilution methods is not a universally applicable method for determining the ability of soils to provide phosphate to plants. In consequence the description of Larsen's parameter as the "*A*" value may be misleading. None the less the value of tracer methods for certain purposes is apparent. The linearity of the relationships between both *E* and *L* and plant absorption in fractions of the same soil enriched with phosphate to varying extents, indicates that the absorption of phosphate from soils of similar sorbing capacity can be inferred from measurements of labile phosphate. The fact that the "*A*" value method has been found of value in some field studies (e.g. Dean, 1955) supports this view. A further purpose for which such measurements may be useful is the determination of the relative merits of different phosphate fertilizers by comparing their effects in the labile phosphate in a test soil.

In considering the relative merits of the E and L value procedures RUSSELL *et al.* note that the study of the factors which cause E and L values to differ in certain soils may yield valuable fundamental information. However, both methods normally give sufficiently similar results for them to be regarded as alternative procedures for estimating the responses to added phosphate likely to occur under field conditions. When used for this purpose the methods must be compared in terms of convenience and economy of effort. The measurement of L values involves the growth of plants under carefully controlled conditions and since the method is applicable only when L bears a linear relation to plant absorption it normally provides no more information than can be obtained in exhaustion experiments of the conventional type. Only in highly phosphate sorbing soils, in which intraspecific differences occur, does the obtaining of unique information appear likely to repay the complication of the method. In contrast the determination of E values is a simple laboratory procedure, readily organised in a routine scale. It holds promise of greatly simplifying the evaluation of soils in which the determination of labile phosphate can provide valuable information.

SOKOLOV (1955) has reported the results of experiments which included the determination of L and E values. The former quantity was described as "assimilable phosphate". As already noted he considered it of great importance that the labelled phosphate added to soil for the determination of L should be accompanied by a low level of carrier. One mg P_2O_5 per kg soil was the recommended maximum. He also noted the importance of the careful mixing of the labelled phosphate throughout the soil. He stressed the importance of using low carrier levels because his fertilizer experiments had shown that the absorption of soil phosphate could be increased, decreased or unaffected by the addition of fertilizers. Thus the values for fertilizer absorption obtained by tracer methods were different from those calculated as the difference between absorption from fertilized and unfertilized soils. These differences were most marked when the original level of assimilable phosphate in the soil was high. In one such example the tracer methods indicated that fertilizer absorption was over 15 times greater than the increase in plant uptake induced by the fertilizer application. The addition of the fertilizer has therefore markedly depressed the absorption of soil phosphate. On the other hand when soil of low phosphate status was used the increase in plant uptake induced by the application of phosphatic fertilizers was 10% greater than the amount of fertilizer absorbed as measured by the labelling procedure; the addition of fertilizer had therefore increased the absorption of soil phosphate. If the level of carrier phosphate added to soil for the determination of L values had comparable effects on the amount of soil phosphate with which the tracer equilibrated the measurements of L would be invalid. There appears as yet however to be no direct evidence to support SOKOLOV's suggestion; the finding of RUSSELL *et al.*, already cited, that L values were unaffected when the carrier level was varied by a factor of exceeding 10^3 is evidence in the opposite direction.

SOKOLOV also reported measurements of isotopically exchangeable phosphate. Five soils were employed, they were treated with 100 mg labelled P per kg soil in the presence and absence of 900 mg carrier phosphate. In addition to measurements of isotopic exchange and plant absorption acetic and carbonic acid extracts were made. The results (Table IV) indicate that when different soils are compared the exchangeable labelled phosphate is not correlated with the absorption of plants, a conclusion similar to that of RUSSELL *et al.* It is of interest that whereas the addition of inactive phosphate to the soils markedly increased the extraction of labelled phosphate by solvents the exchangeable labelled phosphate was little affected.

Table 4—Fixation of Phosphates by the Soil

(SOKOLOV, 1955)

Phosphorus content of different extracts in mg P_2O_5 per kg of soil and supply of phosphorus from the fertilizer.

Soil	mg P_2O_5 introduced	Extracts					Labelled phosphorus taken up by wheat crop
		Carbonic Acid		Acetic Acid		Phosphoric acid (exchange labelled phosphates)	
		Total	Labelled	Total	Labelled		
Thick chernozem	100^+	25	20	61	47	56	20·1
	$100^+ + 900$	442	43	734	63	61	—
Typical sierozem	100^+	32	23	68	61	64	19·2
	$100^+ + 900$	585	61	824	78	47	—
Podsolized soil	100^+	6	4	35	22	53	12·0
	$100^+ + 900$	217	21	447	44	51	—
Podsolized sandy-loam	100^+	4	3	51	34	53	3·8
	$100^+ + 900$	207	18	622	55	42	—
Red soil	100^+	—	—	2	1	47	1·3
	$100^+ + 900$	4	—	87	7	49	—

THE ASSESSMENT OF THE RESPONSE OF CROPS TO PHOSPHATE FERTILIZERS

The results so far considered indicate that once phosphatic fertilizers have entered the soil they cannot be regarded as retaining a separate identity. The effect of their addition on crop growth is not dependent on the amount of fertilizer which enters the plant but on the change in total plant uptake, be it from the soil or from the fertilizer. The realization of this fact has caused the main emphasis in much of the more recent tracer work on crop responses to be diverted from the simple estimation of fertilizer absorption with which many of the early investigations were closely concerned. The study of fertilizer placement, of the effect of grain size and of the relative ability of different parts of the root system to absorb phosphate have received emphasis.

Reviewing Russian investigations KURSANOV (1955) has referred to investigations by RATNER and others on the effect of different methods on fertilizer placement. Advantage was found when the fertilizer was immediately below the seeds. The same workers had also shown that when a small part of the root system was exposed to an ample supply of phosphate its rate of absorption became 20 or 30 times greater than the normal rate and thus the contact of a few isolated roots with ample sources of nutrients could satisfy to a considerable degree the entire requirement of the plant. KLECHKOVSKI (1955) described investigations, mainly using P^{32}, on the effects of grain size, placement and the time of fertilizer application on absorption by plants. In experiments with millet in a podsolized soil it was found (Table 5) that whereas at an early stage of growth absorption was greater from finely divided grains of superphosphate the reverse was true after several weeks. In other experiments the relationship between the depth at which fertilizers were placed and absorption had been examined at different periods using oats as the test plant (Table 6). The advantage of shallow planting for early absorption and of deeper planting for absorption later in the life history of the plant led to the suggestion that the application of fertilizer partly with the seed and partly at the depth of 18 to 22 cm would be superior to the application of the total dose at one depth. This was confirmed in a further experiment with oats (Table 7). In a number of experiments the effects of other nutrients, as well as lime and humus, on the absorption of phosphate were investigated. Striking results were obtained in an acid soil (characteristics not given) in which the addition of lime led to a fifty-fold increase in the absorption of phosphate placed 12–14 cm below wheat seed.

Table 5—Content of Labelled Phosphorus Assimilated by Plants from Fertilizer (*in milligrams* P_2O_5 *per* 100 *plants*)

(KLECHKOVSKI, 1955)

Dates of sampling (in days after germination)	Grains 2 × 2 mm	Grains 4 × 4 mm
10	1·5	0·6
20	5·0	1·2
30	19·5	10·8
41	28·2	33·0
57	99·8	164·0

Table 6—Amount of Phosphorus Assimilated by the Oat Plant from the Fertilizer, Depending upon the Depth of Application of the Fertilizer (*in milligrams* P_2O_5 *per* 100 *plants*)

(KLECHKOVSKI, 1955)

Dates of sampling (in days after germination)	Depth of application of superphosphate	
	7–8 cm	18–22 cm
7	7·1	0·7
14	10·5	11·5
45	60·7	313·7

In the United States extensive investigations have been carried out in similar and related fields. The present summary is abstracted from DEAN's detailed review (DEAN, 1955):

A number of crops growing under a variety of conditions has been studied, including cereals, pasture species, potatoes, sugar beets, sugar cane, tobacco and cotton. With few exceptions, the results of these studies have followed a rather typical pattern. Phosphate fertilizers are usually applied at the time of planting and localized in the proximity of the seed. In the early stages of growth, seedlings absorb phosphorus at a faster rate from the fertilizer than from the soil, but as the plant develops and the root system enlarges, the amount of soil phosphorus accessible to it increases. Thus the absorption of soil phosphorus increases as compared with fertilizer phosphorus. The net result is that young plants characteristically have a higher percentage of phosphorus in the crop derived from the fertilizer than mature plants. The extent of this effect varies between plant species. Relatively little decline in the rate of fertilizer to soil phosphate has been observed in potatoes as compared with corn. This difference between crops is attributed to differences in root systems and in their development.

Table 7—Amount of Phosphate Assimilated by Oat Plants Subjected to Different Fertilizer Treatments (in milligrams P_2O_5 *per 100 plants)* (KLECHKOVSKI, 1955)

Treatments	Amount of labelled superphosphate			Assimilation of phosphorus by the plants 45 days germination (in mg per 100 plants)		Weight of 100 plants in g
	at a depth of		In rows with seeds			
	7–8 cm	18–22 cm		From fertilizer (labelled)	From soil (not labelled)	
1	3 centn/hect	—	—	60·7	1,563	193
2	—	3 centn/hect	—	313·7	1,719	270
3	—	2·5	0·5 centn/hect	406·3	2,248	323

Crops utilize phosphorus with low efficiency from applications of phosphatic fertilizers. The experiments which compare the phosphorus absorption by crops also supply information relative to the efficiency of fertilizer utilization. The percentage of the phosphorus in a given application which is absorbed and thus utilized by the crops grown during the first season has been found to vary between the limits of 2 and 35%. With increasing rates of fertilizer application the total amount of fertilizer phosphorus absorbed usually increases, but the efficiency of utilization usually decreases. DEAN discusses the question: Does the application of fertilizer phosphorus influence the amount of soil phosphorus absorbed by plants? With many soils the addition of fertilizer phosphorus has little effect on the amount of soil phosphorus absorbed. Other experiments have shown that the addition of fertilizer phosphorus actually decreases the amount of soil phosphorus absorbed.

DEAN also reviewed the results of experiments on the placement of phosphate fertilizers. The most pronounced effects of placement of superphosphate on the utilization of fertilizer phosphorus were observed during the early stages of growth. The closer this material was placed to the seed, the greater its utilization. The pronounced differences observed during the early stages of growth largely disappeared as the crop matured. Broadcast applications are the most convenient method of applying fertilizers to pastures and other established stands of crops. Since it is known that phosphates penetrate soils only to a limited degree, this method has not been considered as a very satisfactory means of applying phosphate fertilizers. The top-dressing of established meadows using P^{32} has however been found to be surprisingly effective.

The utilisation of added phosphate has been shown to be affected by liming, nitrogen fertilization and irrigation. Liming of soils above pH 6·5 reduces phosphate absorption and the mixture of phosphate and nitrate has yielded higher uptake than when either is separately applied.

Apart from phosphorus^{132}, calcium^{45}, cobalt^{60} and zinc^{65} have been employed in plant nutritional experiments. KEDROV-ZIKHMAN (1955) has described experiments with Co^{60} which suggest that the addition of that element increases crop yields especially in limed soils in which the availability of cobalt is low. The concentration of cobalt is highest in roots and least in seeds. Both stimulations and depressions of respiration and catalase activity are reported.

MITSUI (1955) has reviewed recent Japanese investigations. In rice simultaneous upward and downward translocation of phosphate has been demonstrated. The effects of H_2S in inhibiting these processes has been emphasised and it is considered that in lowland rice areas the production of H_2S from fertilizers containing sulphate is a major cause of low productivity. Investigations both in the laboratory and in acid soils have led MITSUI to conclude that phosphorus may be absorbed by a "contact solution mechanism" from sources which are insoluble in water but soluble in 2% citric acid. The stimulation of phosphate absorption by the additions of nitrogen has also been observed.

Whereas the majority of tracer investigations on nutrition have been carried out with annual or biennial species the groups of workers associated with both Spinks (1955) and MITSUI (1955) have concerned themselves also with nutrition of trees. SPINKS reports investigations of the distribution of P^{32}, Ca^{45} and Rb^{86} in forest trees. The maximum rate of upward movement of Rb^{86} observed is 30 cm per minute. When only part of the root system has been supplied with labelled phosphate the radioactivity was not uniformly distributed between branches. This suggests that certain roots provide nutrients to certain branches and not to the entire canopy. MITSUI, discussing the work of USHIDO on the phosphate nutrition of mulberry trees, emphasizes the importance of applying the nutrient in close proximity to roots on account of the slow movement of phosphate through the soil.

THE MOBILITY OF IONS IN SOILS

DEAN (1955) included in his review a discussion of work on the mobility of ions in soil.

The earliest report on the use of radioisotopes to study movement in soils was that of HENDERSON & JONES (1941). Monocalcium phosphate labelled with

P^{32} was placed on the surface of soils and water was then added to simulate 2·5 inches of rain. Movement of phosphorus varied from 1·25 inches in a Cecil clay to 4 inches in a Crosby silt loam. Not more than 5% of a potassium application labelled with K^{42} moved over 1·6 inches.

The movement of phosphate ions away from the site where they are introduced into a soil is considered to be limited by the phosphate fixation processes. Ordinary chemical methods do not permit a study of this movement in adequate detail because of the relatively large quantity of phosphorus already present in most soils. Through the use of radioactive phosphorus it has been possible to trace the movement of phosphatic fertilizers under actual field conditions. When 2 300 lb. P_2O_5/acre of P^{32} labelled phosphoric acid was added to a soil in conjunction with 4 inches of irrigation water the phosphorus penetrated about 12 inches and the water about 20. After 11 and 43 days, 99 and 86% respectively of this phosphorus was in the first 6 inches. The movement of phosphorus from the superphosphate was much less than that from the liquid phosphoric acid when both are applied in bands to irrigated soil. Very little superphosphate moved beyond 3 inches. The phosphoric acid moved to a depth of 12 inches, but 85% of the total application was found in the top 4 inches. In other investigations barely 5% of the added phosphate moved more than one inch. Increasing increments of irrigation water cause greater downward movement of superphosphate applied to the surface of the soil. The extent of diffusion of phosphate from different fertilizer materials had been shown to depend on the fraction of the fertilizer phosphorus in water-soluble form. The distance of diffusion increased with time and with the rate of phosphorus application. In 4 weeks the fertilizer phosphorus diffused only 3 to 4 cm. Under otherwise uniform conditions, diffusion of fertilizer phosphorus differed between soils and was considerably less in calcareous soils than in acid soils. The rapid dissolution of phosphorus from granular superphosphate in contact with moist soil had been demonstrated. Even in soils as low as 2 to 4% moisture, 20 to 50% of the phosphorus moved from the granules to the soil in one day.

The leaching of calcium in soils and its relation to problems of soil acidity has been investigated using Ca^{45}. Calcium had been shown to move downward in the soil by a continuous series of displacements, the calcium appearing in the leachate being that originally present in the lower layers of the soil. Lysimeter studies using Ca^{45} labelled calcium carbonate and a calcium silicate slag had revealed a somewhat complicated picture due to differences in the rates of dissolution of the added calcium and incomplete isotopic exchange with the native soil calcium.

PHYSICAL PROPERTIES OF SOILS

VOMOCIL (1955) described the use of gamma rays from Co^{60} to measure the bulk density of soil. The conductance of X-rays and gamma rays through matter accords with the Beer-Lambert Law

$$\frac{I}{I_0} = Ke^{-ux}$$

when I is the intensity of radiation transmitted by a thickness x, I_0 the initial intensity, and u, the linear absorption coefficient, of the medium. Thus it is possible to infer the density of material for the extent to which it transmits γ radiation. The apparatus employed by VOMOCIL consisted of a one millicurie source of Co^{60} mounted approximately 30 cm distant from a Geiger-Muller tube connected to a rate meter. The form of the apparatus was such that the Co^{60} source and the counter could be lowered into parallel holes bored in soil. In practice, it was found that the relationship between the observed counting rate and the wet bulk density of soil was not linear as was expected on the basis of the Beer-Lambert Law (Fig. 4). This effect, which was attributed to changes in geometry with density, did not affect the practical value of the method.

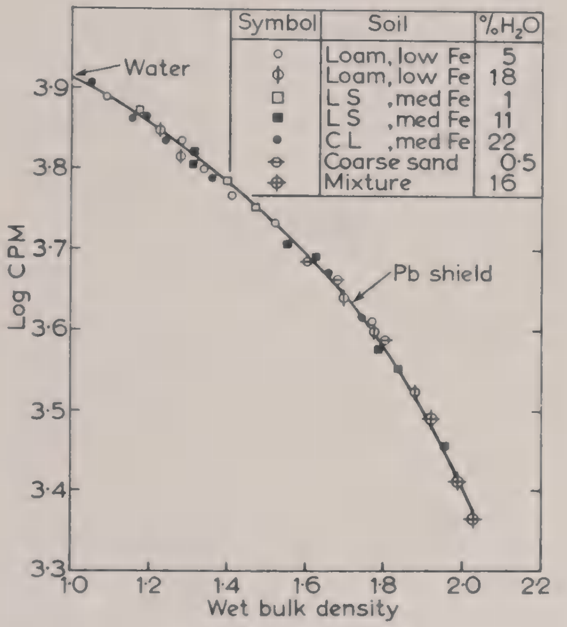

Fig. 4. Calibration curve for densitometer.

Discussing the results so far obtained, VORMOCIL states:

"The method proved accurate and rapid in field trials. Readings ranged from 2 000 counts per minute for a wet bulk density of 2, to 8 600 counts for a density of 1. Values obtained with this method were, in general, about 1·5% lower than those found with the core sampler technique on the same soil. Under good conditions a crew of three could make 200 measurements a day with much less effort than would have been required with former methods and with much less plot disturbance. This rate was possible with some measurements being made at depths of 50 cm in the soil.

"The most reliable interpretation of results was possible on a relative basis. That is, where two areas of soil similar in texture, horizon development and location, drainage, and depth were available, one under good management, the other under poor management, comparison of the density profiles from the areas indicated the magnitude and position of important differences in soil compactness.

"Where such comparisons were not possible, soil air space, calculable from bulk density and soil moisture data was a useful index for evaluation of soil physical condition. Significant correlation was found between yields of potatoes and corn and the average bulk density of the 10 to 40 cm depth region. This occurred only at relatively high densities (1·50–1·70) for loams. At lower average profile densities, correlations were not significant. The relationship between relative yield and average profile density appears to be parabolic, indicating that density enters twice as a factor in productivity. Significant decreases in yields of potatoes and corn occurred only after air space fell to about 12–14%."

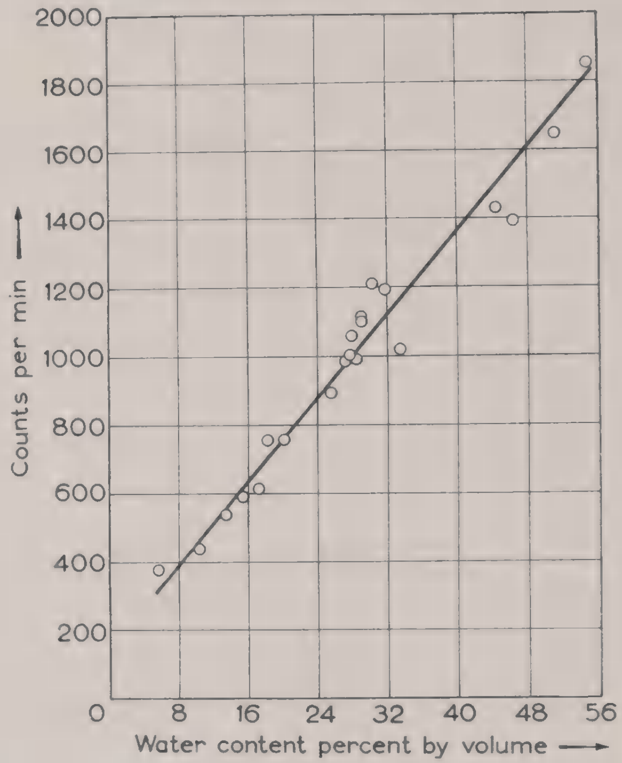

Fig. 5. Calibration curve for neutron moisture meter using a 50 mc radium beryllium source. (Spinks, 1955.)

Spinks (1955) has discussed the advantages of employing neutron absorption to measure the moisture content of soils: "The measuring of changes in soil moisture in a given sample of soil involves using a non-destructive method of sampling, and heretofore no completely satisfactory method has been available. The neutron method seems to offer certain advantages over those previously suggested in that it is non-destructive, is adaptable to all types of soils, is independent of the state of the moisture (whether vapour, liquid or solid), is able to indicate rapid changes in moisture content, and is relatively independent of salt content of the soil. In the neutron method, fast neutrons emitted by a neutron source are scattered by the atoms in the soil, thereby losing their energy and becoming slow neutrons (apart from loss by capture). The slow neutrons are then measured by means of a slow neutron detector such as an indium or rhodium foil or a BF_3 chamber. The fractional loss in energy when a neutron collides with an atom is at a maximum for hydrogen, and thus soils with a high moisture content are particularly effective in slowing down neutrons. The activity induced in a slow neutron detector is correspondingly high and is, in fact, proportional to the moisture content of the soil when standard conditions of neutron irradiation are adopted. It can be shown theoretically that the distribution of slow neutrons about a point source of fast neutrons is a function of the ratio of the slowing down length to the diffusion length. The slowing down length is defined as the average distance a neutron must travel in a medium before it is slowed down to thermal velocity. It is made relatively small by hydrogen in comparison with other common elements in soil. The diffusion length is one-sixth the average distance from point of origin to point of capture of the neutron. It does not vary, with changing moisture content, as strongly as does the slowing down length. The net effect is that if a slow neutron detector is placed near a source of fast neutrons in soil, the activity will be largely a function of the hydrogen (or moisture) content of the soil."

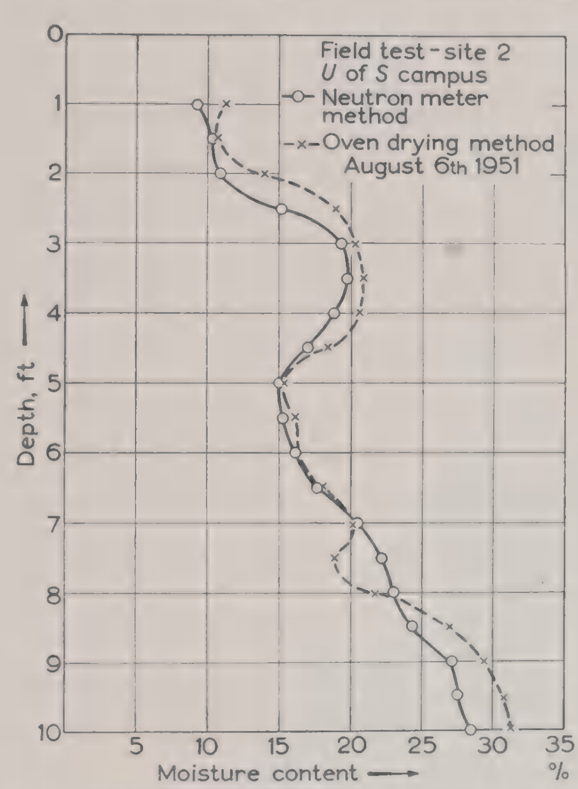

Fig. 6. Moisture profile comparison between standard oven-drying method and neutron meter. (SPINKS, 1955.)

An apparatus suitable for field trials has been described (LANE *et al.* 1952). It embodies a 50 mc Ra-Be neutron source and indium or rhodium foil as a detector. The apparatus is so designed that it can be lowered into a hole 5 inches in diameter. After the detector has been exposed for a fixed time it is removed and the induced activity is determined with a portable beta-gamma rate meter.

It has been found of advantage to collimate the neutron beam using hollow cadmium cylinders filled with paraffin.

SPINKS (1955) reported the results of a field experiment: "A series of holes, six feet deep and two inches in diameter, were drilled at various levels on the banks of the South Saskatchewan River to give a variety of soil types, densities, and moisture contents. The soil types encountered in these holes ranged from silts to medium plastic alluvial clays. The holes were drilled with a 5 cm auger and lined with 5 cm diameter aluminium pipe (0·125 cm thick). The depth of the source could be varied by adding lengths of aluminium tubing to the source holder and lowering it down to the desired depth in the hole. Samples of the soil were taken every 15 cm, during excavation of the holes, for determination of moisture content by the standard oven-drying method. At each depth, three readings for moisture content, using the rate meter, were averaged to give a mean value. This was plotted in Fig. 5 against the average measured moisture content in the 15 cm above and below the source, since the zone of influence of the source is primarily a sphere of about 15 cm radius. Fig. 5 shows a reasonably linear relation between count rate and mass of water per unit volume. This curve was used as a standard calibration curve for subsequent tests. Fig. 6 shows a comparison of moisture content determinations in a vertical hole on the campus of the University of Saskatchewan for the neutron meter and the usual oven-drying method. All determinations were within 3% moisture, and most were within 2% moisture or less."

III. THE ENTRY OF NUTRIENTS INTO PLANTS THROUGH STEM, LEAF, AND FRUIT, AS INDICATED BY RADIOACTIVE ISOTOPES

THE principal mineral nutrient absorbing organ of the plant is the root. That other organs and parts such as stems, leaves, flowers, and fruits might also be able to take up nutrients has long been indicated by certain horticultural practices (BALLARD & VOLCK, 1914; FORSYTH, 1803). Yet only recently, largely with the aid of both radioactive and stable isotopes, has this truth been demonstrated experimentally. The phenomenon has been variously termed "foliar feeding," "uptake by above-ground or aerial parts" (TUKEY *et al.*, 1955a) and "non-root nutrition" (KURSANOV, 1955).

ENTRY OF NUTRIENTS INTO BRANCHES

Radioactive minerals have been shown to enter even through the bark of fruit trees. Cotton gauze was dipped into solutions of K^{42} labelled potassium carbonate and P^{32} labelled ortho-phosphoric acid, and the impregnated gauze wrapped around branches of apple (*Malus domestica*) and peach (*Prunus persica*) trees (TUKEY, 1952). Within 24 hours of application, even during winter with temperatures below freezing, radioactivity was detected within the dormant branches 18 to 24 inches both above and below the points of application. Similar applications made in the spring just as the buds were commencing to swell showed the material to move in through the bark and up through the branches, and to concentrate near the buds, available for the flush of new growth.

THE LEAF AS AN ORGAN OF ABSORPTION

Until recently, the general conception has been that leaves are covered by a continuous, impervious coating of cutin (McDANIELS & COWART, 1954). However, in 1948, ROBERTS, SOUTHWICK and PALMITER (1948) reported that microchemical examination of apple leaves (*Malus domestica*, var. McIntosh) indicated a relationship of cell wall constituents to penetration of soluble materials applied to the leaf surface. They showed that there is by no means a continuous covering of an apple leaf by cutin, and that the layering of cell walls is not as a uniform, solid structure. Instead, pectinaceous substances are to be found in intermittent parallel layers in the outer walls of epidermal cells, interspersed with cutinized areas. Such pectinaceous materials form a continuous pathway extending from outside the leaf to the walls of vein extensions and from there directly into the vascular system of the leaf (WYLIE, 1943). In a study of the interaction of isolated cuticles of *Prunus armeniaca* with various foliar applied elements, ORGELL (1955) considered their sorptive properties as resembling semi-lipoidal cation exchange membranes.

Also, LAMBERTZ (1954) has recently re-emphasized the importance of plasmodesma. He has found 2–200 to 60–800 protoplasmic strands per 50 square microns in the walls of epidermal cells, and concludes that there may be 8 000 to 9 000 such strands in the outer wall of a single epidermal cell. It appears that these strands of protoplasm end directly under the cuticle.

Accordingly, the leaf is structurally well equipped to absorb materials through both the upper and lower surfaces. In addition, the leaf area of a plant is considerable. Thus, it has been determined that a 12-year-old apple tree provides in its foliage, both upper and lower surfaces, an area of one tenth of an acre or approximately ten times the spread of the tree (TUKEY, 1952).

FACTORS AFFECTING ENTRY OF NUTRIENTS INTO LEAVES

It may be anticipated that foliar absorption of nutrients will be affected by external and internal factors. Temperature, light, humidity, the pH and carrier ion of the treating solutions, and various additive chemicals may be important, as well as the species of plant involved, the morphological nature of the absorbing organ, and the nutritional status of the plant (COLLANDER, 1941).

Effect of Temperature and Humidity

Under controlled conditions, the uptake of phosphorus increases directly with rise in temperature (LONG *et al.*, 1955; TUKEY *et al.*, 1955a, b). Some instances have been reported of reductions in uptake at higher temperatures, but this reduction is associated with an accompanying lowering of humidity, drying out of foliage, and reduced solubility of materials deposited on the leaves (KAINDL, 1954). From detailed studies of plants growing near the site of an atomic blast in an arid climate, it was concluded the mechanism of absorption of fission products was through airborne soil which lodged upon the leaves and was then dissolved in nocturnal dew (RUSSELL *et al.*, 1955).

Effect of Stomatal Opening, Light, and Addition of Sucrose and Boron

Opening and closing of stomata is of little significance, since foliar absorption of nutrients is not confined to daylight hours. For foliar applied N^{15} labelled urea, uptake at night was three to ten times that during the day (VOLK & MCAULIFFE, 1954). Initially, light has a slight but significant effect in increasing foliar absorption of radiophosphorus, radiopotassium and radiorubidium by bean leaves, but this initial stimulation disappears and the actual accumulation of foliar-applied radionuclides by roots and buds is favoured by prolonged darkness. The initial depression of absorption in the dark may be duplicated by the addition of sucrose to the treating solution. An effect similar to that obtained with sucrose occurs when boron is added to a phosphate solution. Not only does borate (0·005%) depress the initial absorption of phosphate, but this initial depression is independent of temperature. In contrast, YATAZAWA & HIGASHINO (1952) found with young wheat plants that, if phosphorus was applied together with sugar (5% solution) on the leaf surface, phosphate uptake was increased threefold.

Effect of Leaf Age, Vigour, and Portion Treated

Young and rapidly expanding leaves are more efficient in absorption than are leaves which are fully matured (FISHER & WALKER, 1955), although all functional green leaves seem to serve as organs of absorption of materials applied externally as sprays or dusts. Maximum uptake is associated with "maximum vitality" of the plant (KAINDL, 1954). Both sides of the leaf blade, as well as the petiole, function in absorption (TUKEY *et al.*, 1955b). Greater entry of P^{32} has been reported through the lower than the upper surfaces of *Solanum nigrum* leaves, but the results are not directly comparable since applied droplets covered a 50% greater area on the under side of the leaf than similar droplets placed on the upper side (KAINDL, 1954). On the other hand, critical studies with primary leaves of the bean plant (*Phaseolus vulgaris*) have shown slightly greater absorption through the upper than the lower surfaces, as indicated by the accumulation of radioactivity in the roots following applications of radioactive phosphoric acid solution (TUKEY *et al.*, 1955b). Since the frequency of stomata is seven times as great in the lower epidermis of the bean leaf (ECKERSON, 1908), it would appear that phosphate entry is not facilitated by these structures.

Placement of the treating solution at the tip, along the outer margin, and in the centre of bean leaves along the midrib, has shown only a slight increase in absorption when compared to similar positions on the lower surface. On the other hand, application to the petiole of the leaf has resulted in very rapid uptake. However, the region of placement on the leaf surface has been found to be of no importance in determining the final level of phosphorus which accumulates in the roots 12 hours or more after treatment (TUKEY *et al.*, 1955b).

Effect of Wetting Agents and Detergents

Leaf absorption of mineral nutrients may be either facilitated (FISHER & WALKER, 1955) or impeded (SWANSON & WHITNEY, 1953) by the addition of a wetting agent or detergent to the formulation.

Effect of pH and Ionic Carrier

Ammonium, potassium, and sodium phosphates are absorbed less effectively by bean leaves than orthophosphoric acid to which only a slight amount of ammonium, sodium, or potassium has been added (pH 2–3). Uptake of the latter was two to ten times that of the former (TUKEY *et al.*, 1955b). Similar results with other crops have been reported (FISHER & WALKER, 1955; NORTON, 1954). By contrast, the absorption of rubidium was accelerated ten to twenty times at pH 8, compared with pH 4 when phosphate was the carrier. At similar pH levels and with the same carriers, potassium uptake when applied as the citrate at pH 8 was twice that of other forms (TUKEY *et al.*, 1955b).

Mechanism of Entry into Leaves

The many factors known to influence the absorption of radionuclides applied to the surfaces of aerial plant parts provide evidence for the existence of more than a mere passive entry through these surfaces into the plant. The effects of pH and cation carrier, when considered in the light of the ionic species and the degree of molecular dissociation which occur, particularly with phosphate, suggest an exchange mechanism in the entry of foliar applied radionuclides. This is confirmed by the apparently competitive action of the borate ion (TUKEY *et al.*, 1955b). That the exchange is active rather than passive would seem to follow also from certain temperature relationships (ASEN *et al.*, 1953, 1954). Ionic penetration would preclude diffusion through the cuticle, and the now demonstrated imbrication of the cuticle (ORGELL, 1955; ROBERTS *et al.*, 1948) with pectic substances and the widespread occurrence of epidermal plasmodesma (LAMBERTZ, 1954) provide most probable sites for protoplasmic exchange at the surface of the leaf.

COMPARATIVE DISTRIBUTION OF NUTRIENTS WITHIN THE PLANT FOLLOWING APPLICATIONS TO ROOTS AND TO LEAVES

(Figs. 7 and 8)

Such highly mobile elements as nitrogen, phosphorus, potassium, and rubidium applied to aerial plant parts are readily translocated, both acropetally and basipetally, at a rate comparable to that which follows root absorption. Reports are similar for apple trees (*Malus domestica*) (EGGERT *et al.*, 1952), cotton (*Gossypium hirsutum*), and sugar beet (*Beta vulgaris*) (KLECHKOVSKI, 1955), mulberry (*Morus multicaulis*) and rice (*Oryza sativa*) (MITSUI, 1955), chrysanthemum (*Chrysanthemum morifolium*) (ASEN *et al.*, 1953, 1954), poinsettia (*Euphorbia pulcherrima*) (LONG *et al.*, 1955), tomato (*Lycopersicon esculentum*) (SIBERSTEIN & WITTWER, 1951), and bean (*Phaseolus vulgaris*) (SWANSON & WHITNEY, 1953). Foliar applied phosphorus accumulates rapidly in growing meristematic regions (WITTWER & LUNDAHL, 1951; WITTWER & TUKEY, 1954) such as root tips, vegetative growing points, flowers, fruits and seeds, and even embryos within seeds. When P^{32} labelled phosphoric acid was applied to the soil in a band in the root area, and compared with sprays to the above-ground parts during early flowering, it was found that the sprays were far more effective per microgram of applied phosphorus accumulated in the developing tomato fruit. With the bean, the sprays were more than twenty-five times as efficient as the root applications in terms of phosphate uptake based on the percentage applied (McCALL *et al.*, 1955). In fact, up to 95% of the phosphorus applied to tomato foliage may be utilized by the plant (TUKEY *et al.*, 1955b). This represents the most efficient method of fertilizer placement yet devised.

Typically, many crops, especially fruits, flowers, and vegetables, during normal development pass through critical periods associated with flowering and fruiting when nutrient demands are high, and when availability from soil sources is low or at a minimum. It has been demonstrated by the isotope technique that during these critical periods leaves can supplement the function of the roots as nutrient absorbing organs, and accomplish such results as increasing the sugar content of sugar beets (*Beta vulgaris*), improving the set of cotton balls (*Gossypium hirsutum*) (Klechovski, 1955), and increasing the yields of cabbage (*Brassica oleraceae*) and other crops at a time when low soil temperatures are unfavourable to nitrogen uptake (Kursanov, 1955). Up to 25% of the phosphate needed in early fruit growth can be supplied by properly timed foliage spray applications.

In contrast to the free movement of phosphorus with the plant, calcium, strontium, yttrium, barium, and ruthenium do not move freely from the absorbing organ, and basipetal transport is negligible (Johns, 1955; Martin, 1954). Thus, relatively low radio-activity (Sr^{90}, Y^{90}, Ru^{106}, Rh^{105}, Ce^{144}, and Pr^{144}) was found in roots as compared with leaves of plants growing near the site of an atomic blast (Russell *et al.*, 1955). Further, there is little or no movement of Ca^{45} from treated leaves of the strawberry (*Fragaria* spp.) (Norton, 1954). Following application to the roots of the mother plant, active upward transport of Ca^{45} occurred. Lateral movement then followed into the stolons and aerial parts of daughter plants. On the other hand, there was an almost complete absence of basipetal transport into the roots of daughter plants, shown strikingly in autoradiograms. Obviously, as revealed by the radioisotope technique, the roots of the runner plants must depend upon their own absorption for a source of calcium.

The peanut (*Arachis hypogaea*) plant has provided another example of limited calcium movement. It has been demonstrated (Bledsoe, 1949) that a calcium supply is necessary in the pegging zone for normal fruit development. Studies with Ca^{45} show that the calcium absorbed by the roots is not translocated to developing fruits once they are established in the ground, and that the fruit must thereafter depend upon securing its own supply of calcium from the soil.

Results with strontium (Sr^{90}) are similar to those with calcium (Martin, 1954). Slight downward movement of Sr^{90} has been found following foliar applications to bean (*Phaseolus vulgaris*), beet (*Beta vulgaris*), and tomato (*Lycopersicon esculentum*), but the amounts were no more than traces of that applied. Chelation of strontium with ethylene diamine tetracetic acid did not increase translocation away from treated tomato leaves.

Movement of P^{32} and Ca^{45} Across a Graft Union Involving Reversed Polarity

By pruning the tops of rapidly growing tomato plants, lateral shoots were forced from two plants which were then grafted together terminally so that the polarity of the two portions were in reverse position to each other (Bukovac, 1955). To one plant of a grafted combination P^{32} was applied through the roots by addition to the soil medium. Another combination was similarly treated but with Ca^{45}. One hour after treatment, nearly as much P^{32} was found on the non-treated as on the treated side of the graft union. On the other hand, movement of Ca^{45} across the union was effectively blocked. In graft unions which

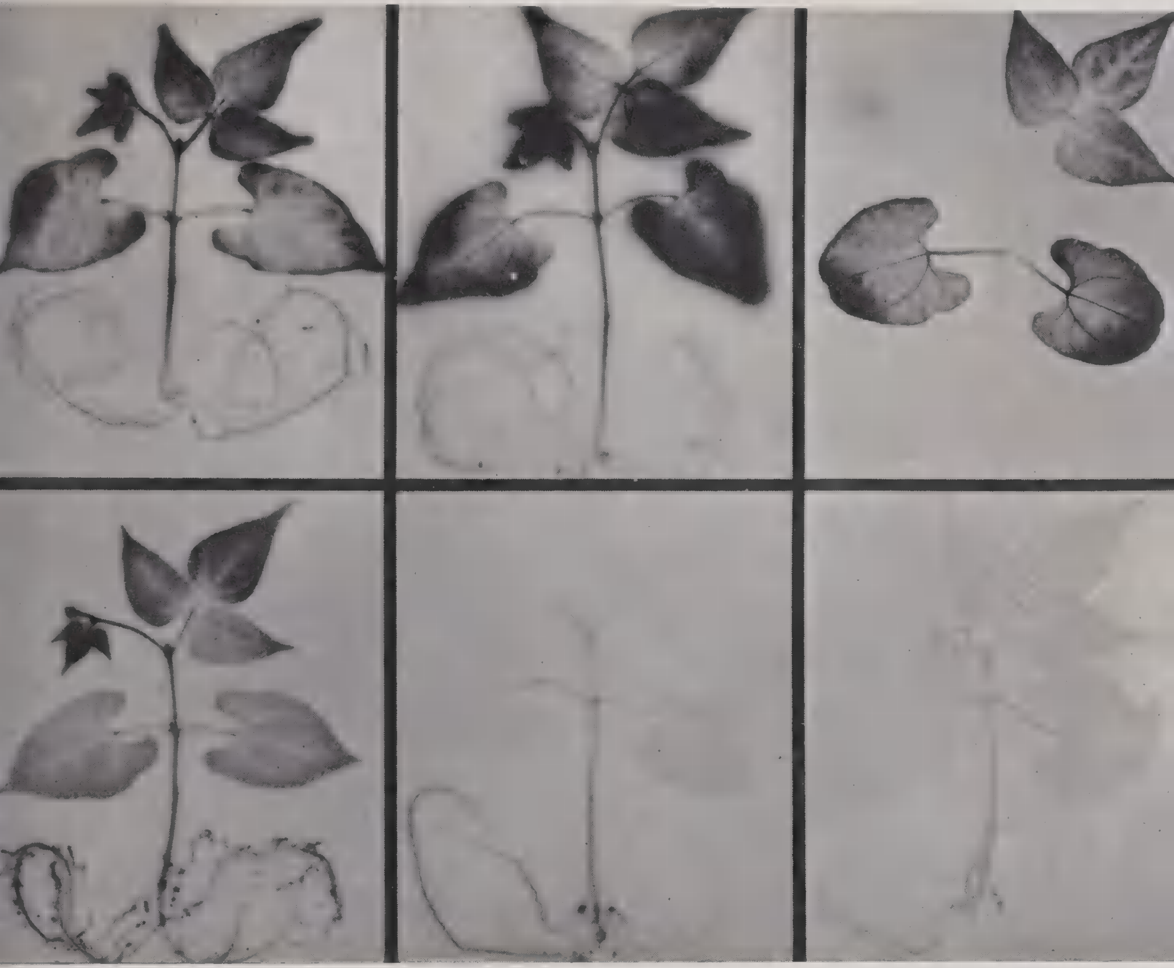

Fig. 7. Autoradiogram showing comparative distribution of P^{32}, K^{42} and Ca^{45} in the bean blant (*Phaseolus vulgaris*) following treatments of roots and leaves.

Top, foliar applications
Bottom, root applications
Left to right, P^{32}, K^{42}, and Ca^{45}.

Note high degree of transport of P^{32} and K^{42} from the leaves to the roots as contrasted with lack of transport of Ca^{45}.

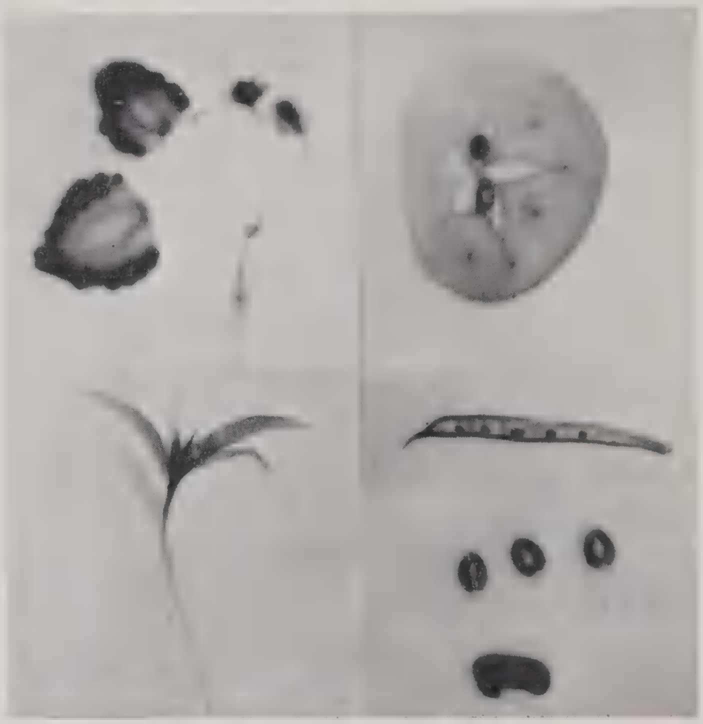

Fig. 8. Autoradiogram showing the pattern of distribution and accumulation of radiophosphorus in the fruit and seeds of strawberry (*Fragaria* spp.), apple (*Malus domestica*), bean (*Phaseolus vulgaris*), and tip of a peach (*Prunus persica*) shoot following foliar application of P^{32} labelled orthophosphoric acid. *Note* the high concentrations in regions of metabolic activity.

did not involve a reversal of polarity, acropetal transport of calcium was not impeded.

ENTRY OF NUTRIENTS INTO FRUITS

Minerals may also enter directly through the epidermis of the fruit (JOHNS, 1955; MARTIN, 1954). When tomato fruits were painted with Sr^{90} strontium chloride and Ru^{103} ruthenium chloride, the radioisotopes were absorbed through the skin of the fruit in considerable amounts but without selective accumulation in any particular tissue. Both flesh and seeds became radioactive. No transport of either Sr^{90} or Ru^{013} was detected out of the fruit into other tissues. Chelation of strontium with ethylene diamine tetracetic acid did not facilitate its transport in the plant.

LEACHING OF NUTRIENTS FROM AERIAL PLANT PARTS

The loss or "leaching" of mineral nutrients from leaves has been demonstrated by the use of isotopically labelled radionuclides. Measured quantities of radiophosphorus (P^{32}) were applied to the roots of bean (*Phaseolus vulgaris*), sweet potato (*Ipomaea batatas*), and poinsettia (*Euphorbia pulcherrima*). After 48 hours absorption, the foliage was leached with no loss of P^{32}. However, if stem cuttings of these plants were supplied P^{32} through the bases of the cut stems and then subjected to foliage leaching, 1·5 to 12·8% of the absorbed P^{32} was lost (SWEET & LONG, 1955).

Results with radiopotassium (K^{42}) and radiorubidium (Rb^{86}) were different. Following a 12-hr. absorption of these ions, subsequent leaching for 4 hours removed up to 71% of the previously absorbed K^{42} and 14% of the Rb^{86}. The loss was greatest from plants grown in darkness at full nutrient level.

FOLIAR APPLICATIONS OF C^{14} UREA

Urea is one of the most useful and well-known nitrogenous fertilizers for leaf application. With some horticultural crops the entire requirement for nitrogen can be satisfied by a few appropriately timed sprays. Radioisotopes provide a specific and sensitive tool to ascertain the rate and extent of utilization, and to predict crop tolerance of foliar applications of urea nitrogen. The first step in the utilization of the nitrogen in urea by the leaves of plants presumably is hydrolysis by the enzyme urease, splitting the urea molecule and giving ammonia and carbon dioxide. As a measurement of urease activity, and thereby the rate of hydrolysis and possible utilization of urea applied to the leaves of horticultural plants, radioactive C^{14} urea may be employed and the rate of evolution of radioactive carbon dioxide determined (HINSVARK *et al.*, 1953).

The apparent relative rates of urea hydrolysis (utilization) of foliar applied C^{14} urea by a number of vegetable and fruit crops have been determined and plotted against field and greenhouse tests of the tolerance of the foliage of the same crops to urea (sprays) in pounds per 100 gallons of water (WITTWER & TUKEY, 1954). From such studies it is possible to predict the tolerance of the leaves of various plant species to concentrations of urea applied as nutrient sprays. It is of interest that the leaves of plants which hydrolyse (utilize) urea most rapidly—cucumber (*Cucumis sativis*) and bean—are those which are the most responsive to treatment and which show the lowest spray concentration tolerance. Conversely, crops showing the greatest tolerance—cherry (*Prunus cerasus*), peach (*Prunus persica*), celery (*Apium graveolens*), and potato (*Solanum tuberosum*)—are those which have the lowest rates of urea hydrolysis or utilization. Intermediate crops are apple (*Malus domestica*), strawberry (*Fragaria* spp.), grape (*Vitis labrusca*), tomato, raspberry (*Rubus* spp.), corn (*Zea mays*), and plum (*Prunus domestica*).

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6

SOME APPLICATIONS OF TRACER TECHNIQUE WITH C^{14} IN PLANT BIOCHEMISTRY

I. PHOTOSYNTHESIS

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II. SYNTHESIS AND METABOLISM OF PLANT PRODUCTS

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I. PHOTOSYNTHESIS

Abstract—The overall process of photosynthesis involves a number of interconnected processes. These processes, which are cyclic with respect to both energy and material, are related at some points to well-known respiratory processes. The carbon-reduction cycle in photosynthesis is now known in detail. All enzymes involved in this cycle have been isolated and the sources of energy required for its operation have been identified in terms of reducing agents and high energy phosphate. These sources of energy are derived ultimately from absorbed light energy which brings about the photolysis of water. Possible mechanisms for this photolysis and for the transfer of energy from the photolysis product to the carbon-reduction cycle are discussed here. Experimental data, in the form of quantum efficiency measurements, are presented and partially confirm the theories proposed for the mechanisms of energy transfer. A diagram of the complete process of photosynthesis containing the several cycles and their relations is presented.

IN recent years it has become increasingly apparent that photosynthesis includes a number of cyclic processes coupled to one another in such a way that there is a continuous flow of energy from one cycle to another, resulting in the conversion of light energy into the potential energy of new chemical bonds. These cyclic processes are similar to better-known cyclic processes occurring during respiration in plants and animals, yet do not seem to be in any instance simple reversals of respiratory cycles. Instead, they appear to have many points of contact with respiratory reactions, including common intermediates and enzymes. These points of contact may well lead to interaction between the two processes that could alter the course of each (CALVIN & MASSINI, 1952). It appears that the organization of the green plant, both structural and enzymatic, may provide mechanisms for partially preventing such interactions where they would prove deleterious to the efficiency of the photosynthetic reaction. At the same time, such interactions may be permitted or even induced when beneficial.

1. FUNCTION OF THE CHLOROPLAST

One such device for isolating photosynthesis from respiration is the chloroplast itself. It appears likely that the entire reaction of photosynthesis, from the absorption of light, carbon dioxide, and water to the formation of various end products, occurs in the chloroplasts. This probability was indicated long ago by the observed formation of starch granules in chloroplasts. (However, starch and other high-molecular-weight compounds are also formed outside the chloroplasts from simple sugars, amino acids, and other low-molecular-weight compounds which diffuse out of the chloroplast.) It has long been known that isolated chloroplasts can, under suitable conditions, retain the ability to evolve oxygen at rates comparable to those of photosynthesis and, at the same time, transfer reducing power to a suitable oxidizing agent. Efforts to demonstrate CO_2 reduction with isolated chloroplasts were generally unsuccessful in the past. However, GERRETSEN (1951) found a decrease in the oxidation-reduction potential in illuminated chloroplast suspensions when they were supplied with carbon dioxide and concluded that there might be an uptake of carbon dioxide, though to only about 3% of the rate of photosynthesis. BOYCHENKO & BARANOV (1954) have demonstrated the incorporation of carbon dioxide into reduced organic compounds by isolated chloroplasts under illumination. This incorporation was determined through use of carbon-14-labelled CO_2, providing a very sensitive method for detecting reduced carbon. This result recently has been confirmed by ARNON *et al.* (1954). The rate of carbon reduction in isolated chloroplasts compared with that in a corresponding amount of photosynthesis in intact leaves was not more than 0·5%, and many of the intermediates of the carbon-reduction cycle have not yet been found. In any event, if it turns out that the rate is small compared with photosynthesis and that only some steps involved in the complete cyclic reduction during photosynthesis take place in the isolated chloroplasts, these facts may be taken merely as an indication that some of the factors involved in the rather complex reduction cycle have been lost from the chloroplasts during their isolation, or that only limited amounts of the necessary enzymes are carried down with them.

The carbon-reduction cycle must be easily inactivated or separated during preparation of isolated chloroplasts. This may indicate that the enzymes involved in this cycle are not part of an organized structure, except so far as the chloroplast structure tends to enclose these enzymes and retain within itself a space with high reducing potential and a high level of adenosine triphosphate.

Thus the chloroplast or its immediate environs seems to be the unique location of all important parts of photosynthesis and provides a container or matrix for the complex system of enzymes and cofactors involved in the carbon-reduction cycle as well as a support for the pigment and enzyme structure which carries out energy conversion and decomposition of water.

2. THE CARBON-REDUCING ENZYMES

Two powerful tools have been brought to bear during the past decade on the problem of the path of carbon dioxide reduction during photosynthesis: tracer elements and paper chromatography. It is unlikely that our present degree of knowledge of carbon reduction would be anywhere near as far advanced had either of these tools been missing. In any event, it is now possible to trace the entire path of carbon reduction from the entry of carbon dioxide into the plant cell to the formation of sugars and other end products (BASSHAM, 1954). The essential steps in this process are:

(a) the carboxylation of a sugar phosphate, ribulose diphosphate, to give two three-carbon molecules, both of which are phosphoglyceric acid;

(b) the reduction (with the aid of ATP) of phosphoglyceric acid by reducing agents formed from water by the photochemical reactions; and

(c) the rearrangement of most of the molecules of the reduction product, phosphoglyceraldehyde, to give (with the aid of ATP) more ribulose diphosphate for continued carboxylation, with a smaller part of the sugar phosphates being drained off as end products.

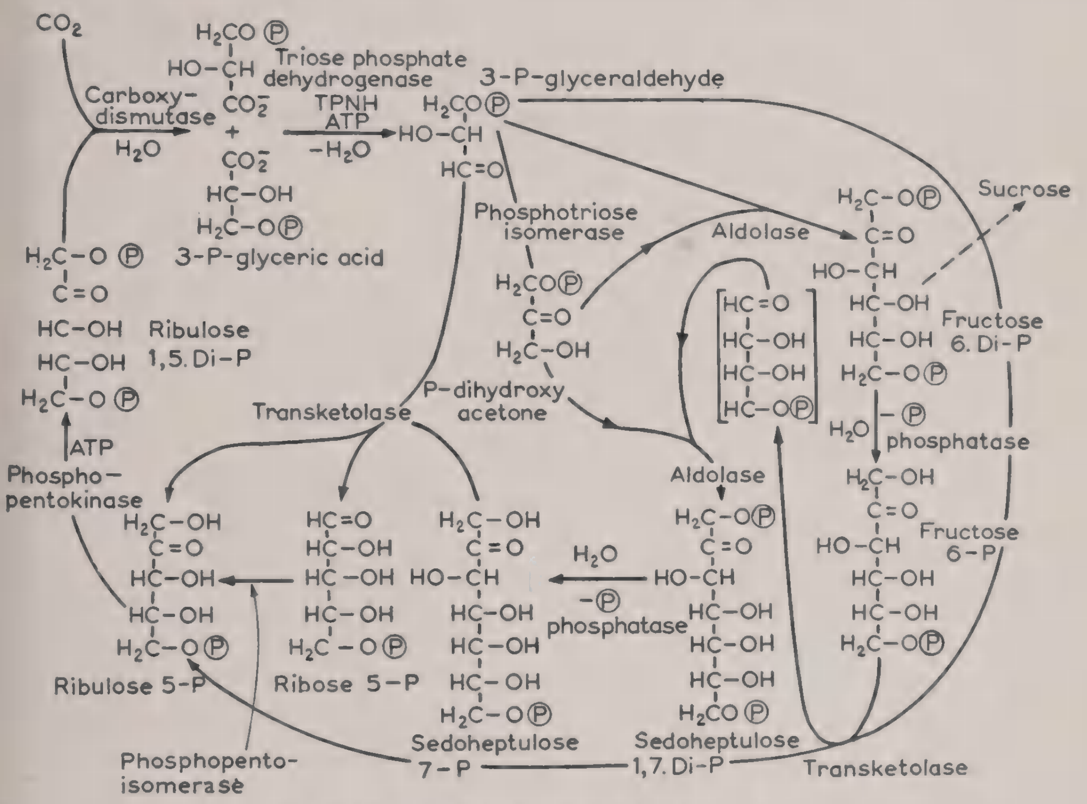


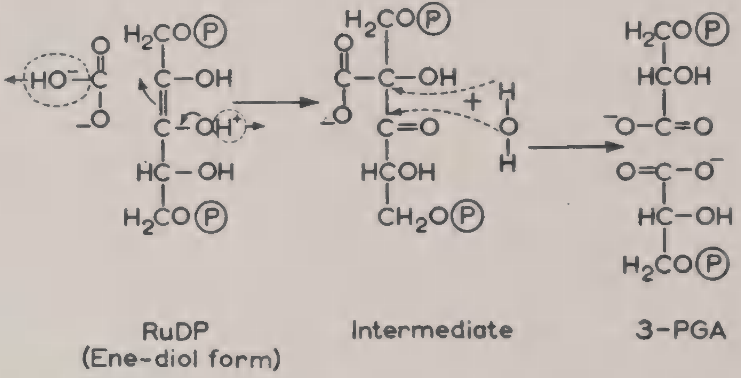
Fig. 1. The path of carbon in photosynthesis.

The individual steps in this process are shown in Fig. 1, together with the enzymes believed to be involved. The carbon balance of this system is indicated in the following scheme.

$$
\begin{array}{lcl}
3\,C^1_5 + 3\,CO_2 & \longrightarrow & 6\ \mathrm{PGA}, \\
6\ \mathrm{PGA} & \xrightarrow[6\ \mathrm{ATP}]{12\,[H]} & 6\,C_3, \\
2\,C_3 & \longrightarrow & C_6, \\
C_6 + 2\,C_3 & \longrightarrow & C_5 + C_7, \\
C_7 + C_3 & \longrightarrow & 2\,C_5, \\
3\,C_5 & \xrightarrow[3\ \mathrm{ATP}]{} & 3\,C^1_5 \\
\hline
12\,[H] + 3\,CO_2 & \xrightarrow{9\ \mathrm{ATP}} & C_3 + 3\,H_2O.
\end{array}
$$

Of all the enzymes indicated in Fig. 1 only two, the enzyme for the carboxylation of RuDP and the enzyme required for the phosphorylation of ribulose monophosphate, have not been found so far in tissues other than green plants. The preparation of the carboxylation enzyme in a cell-free extract has been reported by QUAYLE and co-workers (1954) while WEISSBACH *et al.* (1954) have isolated an enzyme preparation which causes the phosphorylation of RuMP to RuDP. It appears that the phosphorylation and the carboxylation may be unique steps in the carbon-reduction cycle. If these steps are unique to photosynthesis, they may be so only because they either require enzymes not found outside green plants, or because the particular concentrations of metabolites within the chloroplast are required if these reactions are to proceed at a finite rate.

The mechanism of the carboxylation reaction is not known in detail but may occur as indicated in the following scheme:



The formation of two molecules of PGA for each molecule of CO_2 incorporated by photosynthesis by *in vivo* experiments (CALVIN *et al.*, unpublished) has been shown. Earlier enzymatic studies by MAYAUDON (J. MAYAUDON, unpublished results) demonstrated that only PGA was formed from HCO_3^- and RuDP by the enzyme system *in vitro*. The free energy change of this reaction can be calculated as:

$RuDP^{-2}$	+	$HCO_3^- \longrightarrow$	$2\ PGA^{-2}$	+	H^+
$1\cdot10^{-3}\ M$ aq.		$1.4\cdot10^{-3}\ M$ aq.	$1.6\cdot10^{-3}\ M$ aq.		10^{-7} aq.
Ⓟ -170 kcal		-144	$2(-157)$		$-9\cdot6$

$$\triangle F = -10 \pm 5 \text{ kcal}$$

It appears that energy is supplied to the cycle for the reduction of carbon in the form of reduced CO II (TPNH) and ATP, both in the reduction of PGA to phosphoglyceraldehyde, and ATP in the phosphorylation of RuMP. For each carboxylation, using one molecule of CO_2 and producing two molecules of PGA, two TPNH molecules and two ATP molecules would be converted to TPN^+ and ADP + PO_4^- in the subsequent reduction of PGA to phosphoglyceraldehyde. The enzymatic formation of RuDP from ribulose monophosphate has been shown by WEISSBACH *et al.* (1954) to require a molecule of ATP. Since the enzymatic conversion of triose phosphate to ribulose monophosphate probably does not require the expenditure of any ATP or other substances that supply energy through energetic coupling reactions, the net supply of energy to the carbon reduction cycle for each molecule of CO_2 reduced is that required for the formation of two molecules of reduced TPN and three molecules of ATP as follows:

$$2(TPN^+ + H_2O \longrightarrow TPNH + H^+ + \tfrac{1}{2}O_2), \qquad \triangle F = 105 \text{ kcal};$$

$$3(ADP + PO_4{=} \longrightarrow ATP), \qquad \triangle F = +\,32 \text{ kcal};$$

which adds up to 137 kcal. Since the energy required for the reaction

$$CO_2 + H_2O \longrightarrow (C_6H_{12}O_6)_{1/6} + O_2$$

is about + 116 kcal, about 21 kcal apparently have to be expended to make the cycle operate at a high rate. The 137-kcal requirement, on the other hand, represents the absolute minimum of energy that must be obtained from light, exclusive of any losses in the photochemical and other energy transfer reactions. We shall return to a discussion of the energy requirements for these other processes later.

The relation between photosynthesis and respiration is shown in Fig. 2,

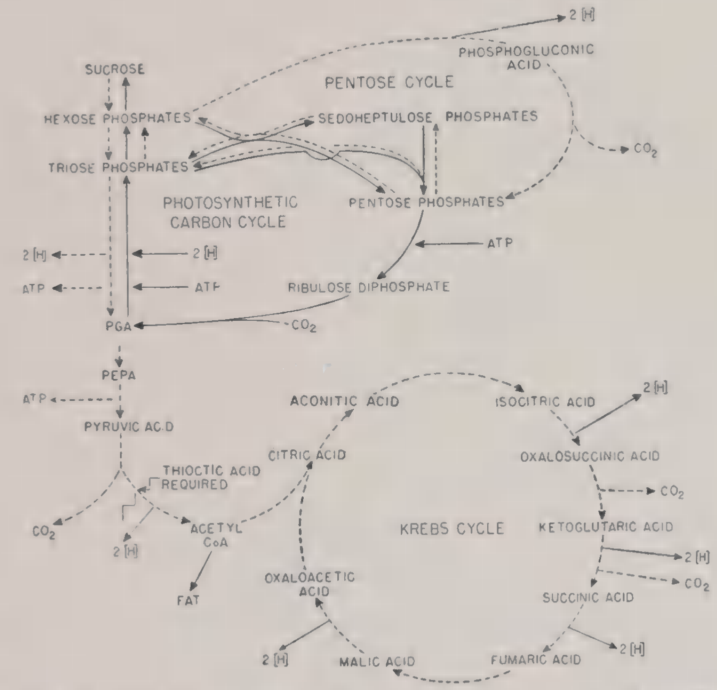


Fig. 2. The relation of carbon paths in photosynthesis and respiration.

where respiration is indicated by dotted lines and photosynthesis by solid lines. The points at which reducing agents and ATP are utilized in photosynthesis or produced in respiration are indicated.

It can be seen that the path of carbon reduction to carbohydrates in photosynthesis is far from a simple reversal of its path in respiration. However, the conversion of PGA to hexose in photosynthesis and the reverse in respiration appear to follow similar if not identical paths. Moreover, some of the pentose-cycle path seems to have some steps in common with photosynthesis.

In addition to the reduction of CO_2 to carbohydrate in photosynthesis it is of interest to consider the incorporation and reduction of CO_2 directly into other organic substances which may be formed in plants. While it has sometimes been implied that carbon reduction goes primarily through carbohydrate metabolism, there is ample evidence that some part of other reduced compounds are formed more directly from carbon dioxide.

In the early phase of our studies we found that carbon dioxide labelled with C^{14} was rapidly incorporated into a number of amino acids (STEPKA, 1948), the most prominent of which were alanine, serine, glycine, aspartic acid and glutamic acid. At the same time, C^{14} was found to be incorporated rapidly into lipid material and into various carboxylic acids of the Krebs cycle. A marked influence of light on the distribution of C^{14} in amino acids formed in plants was observed by RACUSEN & ARONOFF (1954). Further studies of the possible photosynthetic formation of amino acids and proteins were made by a number of other authors (Nos. 6, 7, 9, 19, 11, 12, 13, 14, 15, 16, 17, 21 of NICHIPOROVITCH's paper) and this work was discussed and summarized by A. A. NICHIPOROVITCH (1955) in a paper presented by A. M. KUZIN at the International Conference on the Peaceful Uses of Atomic Energy in 1955. An interesting aspect of these studies was the finding that the amount of C^{14} found in amino acids seemed to be affected by the wave length of light used, there being more C^{14} incorporated into amino acids in blue light than in red light. Since, as will be discussed later, nearly all the absorbed light energy seems to be degraded to the level of red light and transferred via an excited electronic state of chlorophyll a, it would appear that the above effect is a photocatalytic one rather than a significant change in the energy transfer pattern. There remains some question in the above reported work as to whether the experiments with red and blue light were kept on an equivalent basis with respect to intensity of incident light, light absorption or photosynthetic rate.

The Light Reaction

In considering the light reaction, it is necessary to keep in mind the physical arrangement of the chloroplast structure as it is now thought to exist. The fine structure of the chloroplast (or of the grana into which some chloroplasts seem to be divided) is believed to be laminar, with very thin—perhaps monomolecular—layers of chlorophyll alternating with thicker layers of proteins and lipoproteins. While the organization and thickness of these layers seem to vary with the species—and more in some algae than in higher plants—it seems likely that the electrochemical fields that exist at the chlorophyll-protein and lipoprotein interfaces are similar in all cases. THOMAS has recently studied the Hill reaction in particles of sublaminar size (THOMAS *et al.*, 1953). Measurement of oxygen evolution as a function of the particle size showed an ability of particles as small as 10^6 Å^3 in volume to carry out the Hill reaction with about 50% the specific activity of intact grana, but a rapid decrease in such ability with smaller particles, and no activity with particles with volumes of less than 2×10^5 Å^3 at a given light intensity. Higher light intensities, however, resulted in Hill-reaction activity with even smaller particles, so it was concluded that if there is a physical unit of about 10^6 Å^3 volume, or 100 Å diameter, it is capable of producing oxygen in the Hill reaction even if partially fragmented.

Earlier, MILNER *et al.* (1950a) using the photochemical reduction of 3, 6-dichlorobenzene indophenol as a measure of activity, studied the Hill reaction with particles of subgranar size, and obtained activity with a mixture of particles, the majority of which were about 20 Å in diameter. The same workers (1950b) after measuring Hill-reaction activity with these particles (about one-fourth that of intact chloroplasts), found that the particles could be aggregated, by precipitation with a variety of salts in the presence of 15% to 20% methanol, in such a way as to produce particles with increased activity. Loss of lipid material resulted in loss of activity.

These experiments indicate that there are physical units, about 100 Å in diameter and containing about 200 chlorophyll molecules, which are capable of carrying out the Hill reaction nearly as efficiently ($\sim 60\%$) as intact chloroplasts. If these units are broken down further, Hill reactivity falls off rapidly, but is present to some extent with considerably smaller particles, especially if light intensities are high. Moreover, much of the original activity can be restored by reaggregation. The requirements for photodecomposition of water with chlorophyll seem to be some aggregation of chlorophyll, lipids, and protein. RODRIGO (1953) has studied associations of a few molecules of chlorophyll, finding no shift of the red-absorption peak to 6 800 Å. However, when chlorophyll was mixed with some ground-up leaves that contained no chlorophyll initially, some shift of the red-absorption peak toward 6 800 Å and some oxygen evolution in light with quinone were observed. The implication of this result seems to be that a degree of aggregation of the chlorophyll molecules sufficient to shift the red peak as far as 6 800 Å is required before the Hill reaction can function. It is interesting to note in this connection that the absorption spectra of chlorophyll in crystals of varying size formed from ethyl chlorophyllide in acetone has been studied by JACOBS & HOLT (1952) and a shift to the longer wave lengths with increasing crystal size was observed. A maximum shift of the red peak to about 7 450 Å was found, beyond which there was no further shift with larger crystals. This shift is ascribed to resonance interaction between identical chromophores or migration of the resonance energy through the array.

The phenomenon of energy migration through the pigment aggregate brings us to a consideration of the light absorption process. According to present views (DUYSENS, 1951) it appears that most of the energy absorbed by plant pigments for subsequent conversion to chemical energy is either absorbed directly by chlorophyll a or transferred to chlorophyll a. The excited state is presumed to differ in energy from the ground state by only about 40 kcal/mole, corresponding to 6 800 Å light, the longest-wave-length light that brings about photosynthesis with high efficiency. It is postulated that the extra energy that is absorbed at shorter wave lengths, either by chlorophyll a or other pigments, is converted to vibrational energy and eventually lost as heat. Thus the course of energy transfer beyond chlorophyll would be unaffected by the wave length of the light absorbed. It has long been known, in fact, that the yield of oxygen evolved per quantum of light absorbed at least is as high for red light as at any other wave length.

On the other hand, light absorbed at wave lengths around 4 800 Å produces a relatively lower quantum yield indicating that pigments which absorb in that range may transfer their energy to chlorophyll inefficiently or may transfer some of their energy to other chemical reactions inefficiently.

The step in photosynthesis which is perhaps most characteristic is the efficient conversion of energy of an excited state of chlorophyll a to the stored energy of new chemical bonds. The first point to consider is the quantity of energy actually available for transformation. It has been frequently proposed that the primary excited state of chlorophyll a has such a short half life (10^{-13} sec) that direct conversion of the electronic energy of this state to some chemical reaction, or the transfer to some other pigment, might not take place before the energy was lost by fluorescence. Consequently, it was believed that transition to a metastable triplet state with concurrent loss of some of the electronic energy must first occur, followed by conversion or transfer of the energy of the triplet state. According to SCHEIBE (1948), however, transfer of electronic energy in a condensed pigment system can occur in 10^{-14} sec. It seems possible, therefore, that in the aggregated chlorophyll-lipid-protein system, the full 40 kcal may be transferred to a suitable proximate acceptor, at least as far as competition with fluorescence is concerned.

The conversion of electronic energy to chemical energy may involve either (a) the transfer of electronic energy to some acceptor molecule other than chlorophyll, followed by energy conversion, or (b) the transfer of an electron from the chlorophyll aggregate (array) to some other molecule, and a corresponding recovery of an electron by the positively charged chlorophyll-molecule group from some other source. Either of these types of conversion seems possible at the present time, but the latter is somewhat more likely and will be considered briefly.

Of the various possible chemical reactions of chlorophyll under the influence of light we shall consider only the transfer of an electron. Since there is evidence that the light-absorption process functions with chlorophyll in an aggregated system, it is interesting to consider—instead of the reaction of a single chlorophyll molecule—the possibilities that exist with some sort of orderly array of chlorophyll molecules. This array is probably not actually crystalline chlorophyll, but may well be an orderly arrangement of chlorophyll molecules associated with other molecules and protein or lipoprotein. We may think of the electronic system of such an aggregate as a single unit in which the π-electrons of the chlorophyll molecules interact. The absorption of an electromagnetic quantum raises one electron of this system from the ground state, in which it is confined to a single molecule, into a state in which it may migrate throughout the array, i.e., into a conduction level. If there is built into this structure a permanent polar character such as exists at a *p*–*n* junction, for example, these photoconduction electrons diffuse toward the positive end of the permanent dipole, leaving a positive hole to diffuse in the opposite direction.

Thus a separation of charge is induced by the light, which may be neutralized by a suitable electron acceptor at one end and a corresponding electron donor at the other end to drop electrons into the positive holes that have been photocreated. In order to complete the separation of the electron and the positive charge and make use of the electrical energy available, all that is needed is either a semiconductor that transmits only electrons or positive charges, or else an acceptor molecule that can accept either an electron or a positive charge (i.e., contribute an electron) or both, in such a way as to produce an irreversible change.

The possibility of a semiconductor is an interesting one in that it provides a possible function for the lipid constituents. One might consider most of the lipid material as an insulator with occasional conductor molecules to pass electrons through. Such a function might be served by a conjugated molecule like a carotenoid, which could accept an electron at one end and give an electron to a suitable acceptor at the other end. At the same time, the positive charges left behind would be reacting with water, probably through the agency of some metalloprotein, to produce oxygen. The electron acceptor, in the Hill reaction, could be the supplied oxidant such as quinone, while in photosynthesis the acceptor could be the primary carrier of reducing power (thioctic acid).

The advantage of the semiconductor arrangement would be the physical separation of the points at which reducing agent and oxidizing agents are formed. Also the nonspecificity of oxidants required for the Hill reaction could be explained, since they could be reduced directly by electrons supplied by the photoactivation of chlorophyll rather than by some specific primary reducing agent. During actual photosynthesis, a specific reducing agent would undoubtedly be formed and would provide a more efficient transfer of the energy available from the electrons obtained from the photochemical reaction. We would expect that this compound would have special properties that would particularly suit it to the task of accepting electrons and, in its reduced form, transferring energy to the carbon-reduction apparatus. It might be expected that this compound would stimulate the Hill reaction, owing to its special qualifications for receiving electrons. For example, if thioctic acid were the compound receiving the electrons from the chlorophyll during photosynthesis, it might be expected that thioctic acid would stimulate the Hill reaction under suitable conditions even though it is not required for the Hill reaction to function.

Such a stimulation has been observed by BRADLEY and CALVIN (1954), who studied the rate of oxygen evolution in the Hill reaction as a function of added thioctic acid. When quinone was added as an oxidant at a concentration that produced the highest rate of evolution of oxygen with quinone alone, it was found that the addition of thioctic acid in a molar concentration only 0·1 as great as that of the quinone produced a 35% stimulation of the initial rate. Moreover, this stimulation was observed with 6, 8-dithio-octanoic acid (6-thioctic acid), whereas 5-thioctic acid and 4-thioctic acid were not effective. The reduced form (dithiol) of thioctic acid and the more oxidized form (the sulphoxide) were also found to be ineffective. It appears that barring some enzymatic specificity which seems unlikely in the Hill reaction, the special property of 6-thioctic acid that is responsible for its activity is the ring strain in the five-membered ring which permits the more ready breaking of the sulphur-sulphur bond.

Another piece of biological evidence tends to support the suggestion that thioctic acid participates in electron acceptance. It had already been observed that in photosynthesizing plants the conversion of photosynthesis intermediates to Krebs-cycle intermediates is inhibited during illumination, but occurs rapidly in the dark. Since this conversion involves the formation of acetyl CoA from pyruvic acid, a reaction which requires thioctic acid as a cofactor(1), it had been suggested (CALVIN & MASSINI, 1952) that this reaction is blocked in the light because most of the thioctic acid is maintained in the reduced dithiol form within the chloroplast.

One advantage of considering the chlorophyll as an aggregated system is that it permits a more reasonable mechanism for the transfer of one electron at a time, with each electron requiring one quantum of light energy. This corresponds to the well-known four-quantum theory in the primary step, since the transfer of four electrons, requiring four quanta, is required to form one molecule of O_2.

Reactions between a single molecule of chlorophyll, water, and hydrogen or electron acceptor are difficult to formulate, since one is faced in that case with the necessity either of absorbing two consecutive quanta in one chlorophyll molecule in order to have enough energy to form oxygen and reducing agents of the strength of TPNH (about 51 kcal), or else of forming oxygen and a reducing agent of insufficient strength which could be used in subsequent reactions involving dismutations of energy to form better reducing agents. Another alternative is that a single molecule of chlorophyll, activated by one quantum, reacts with some activated, hydrated compound in which the O-H bond could be more readily broken, and thus has left over enough energy to form a good reducing agent. It may well be that water is in fact incorporated into some compound to weaken the O-H bond before the O-H bond is broken, regardless of the mechanism of electron transfer, but it is doubtful if this activation is sufficient to permit the formation of oxygen and reducing power of the strength of TPNH at the same time by one quantum.

Recent proposals involving reactions in which one molecule of chlorophyll provides both electrons in a given H_2O photolysis include the one by LEVITT (1954) who suggests that a chlorophyll molecule, excited by one quantum of light, gives up an electron to thioctic acid forming oxidized chlorophyll, after which the molecule of oxidized chlorophyll absorbs a second quantum and transfers a second electron. The resulting dipositive chlorophyll then reacts with water to produce hydrogen ion and oxygen. In the meantime, the thioctic acid has been reduced by the two electrons to the dithiol.

On the other hand, WESSELS (1954) has proposed a one-quantum-per-two-electrons reaction in which a much weaker reducing agent, reduced Vitamin K, is produced. This reducing agent is then used in part to produce ATP, and in part to produce a reducing agent of the level of TPNH through the expenditure of ATP in a coupled reaction. The formation of reduced Vitamin K and oxygen from water and Vitamin K is said to require 39 kcal, so that the energy of one quantum would have to be used with nearly 100% efficiency. The mechanism proposed by WESSELS for the formation of ATP from reduced Vitamin K is reasonable but energetically very inefficient in that only one ATP is formed for each molecule of reducing agent used. No specific mechanism was proposed for the formation of TPNH from TPN^+ by the oxidation of reduced Vitamin K and the conversion of ATP to ADP and inorganic phosphate, but this reaction would be a bit uphill energetically, since the difference in redox-free energies between TPNH and reduced Vitamin K is apparently about

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(1) BULLOCK *et al.* (1952), GUNSALUS *et al.* (1952), PATTERSON *et al.* (1951), REED & DEBUSK (1952), REED *et al.* (1951).

12·5 kcal, slightly more than the 10 or 11 kcal now thought to be available from ATP hydrolysis. Besides this, the proposal of Wessels requires that the steps in the liberation of O_2 from whatever intermediates may be formed in the reaction of oxidized chlorophyll with water proceed with virtually no change in free energy. In other words, this proposed mechanism includes one very inefficient step and a number of steps that are nearly 100% efficient in energy transfer. While this is entirely possible, we find it slightly more satisfying, from a thermodynamic viewpoint, to suppose that most of the steps involved in the energy transfer from one system to another proceed efficiently but with a small loss of energy in each, thus providing a smooth driving force throughout the entire process which will not require the enzymes at any stage to cope with infinitesimal concentrations of substrates.

Intermediate Transfer Systems

We have already anticipated, in the discussion of both the carbon-reduction cycle and the light reaction, some of the reactions involved in the transfer of reducing power and energy from the light reaction to carbon reduction, and in the evolution of oxygen from whatever products are forming in the breaking of the O-H bond. Assuming, for the time being, that the theories above, which require the absorption of a quantum for each electron taken from water are correct, there is ample energy in four quanta of 6 800 Å light (168 kcal/mole) to bring about the photolysis of two water molecules and the formation of two molecules of reducing agent of strength equal to TPNH:

$$2[H_2O + TPN^+ = \tfrac{1}{2}\,O_2 + TPNH + H^+],$$
$$\triangle F = +\,105\ \text{kcal}$$

The entire excess of energy, some 65 kcal (16 kcal per mole of quanta) minus whatever was lost in the primary absorption and conversion processes, is available for the evolution of oxygen from the intermediates formed from the oxidation of water. The decomposition of water requires by far the greater portion of the energy available from the primary photochemical reaction. From the redox potential of the primary reducing agent, which in our scheme must be about 0·3 V, we can say that the energy stored by the transfer of the electron to the reducing agent is about 7 kcal/mole (taking that in H_2 at one atmosphere in contact with 1 NH^+ as zero). This leaves a remainder, from a 42-kcal/mole-quantum, of about 35 kcal. If there is a loss of about 5 kcal in the transfer of the electron from the chlorophyll aggregate, there is left about 30 kcal/mole to be stored in each positive charge ("hole"). This would then be a potential of about 1·3 V. The potential required for the half reaction

$$2\,H_2O = H_2O_2 + 2\,H^+ + 2^-_e$$

is about 1·2 V at pH 7 and 10^{-6} M H_2O_2. Thus the reaction will go as written provided that the very high activation energy required for the removal of electrons from water-oxygen atoms, which would result in formation of hydroxyl radicals in solution, can be overcome. We may suppose that the hydration of a suitable surface on the granar fragment, perhaps resulting in actual hydrated compounds, results in an orientation of —OH groups which permits the formation of O-O bonds concurrently with the removal of the electrons from the water.

The formation of the positive and negative potentials discussed above requires some mechanism for obtaining just the right distribution of energy to achieve the necessary oxidation and reduction. This can be accomplished by extending somewhat further the proposal for the separation of charges through the agency of semiconductors. We may think of the subgranar unit as a photoelectric battery. The driving force for this battery is the light energy absorbed by the chlorophyll. The absorption of light produces in the aggregate conduction electrons and their corresponding positive "holes". This part of the structure can be considered a conductor after light absorption. On either side of the chlorophyll aggregate is a layer of semiconducting material. This material may be lipid or lipoprotein. One layer contains a permanent structural excess of electrons (n volume), permitting the conduction of positive charges. The other layer contains a permanent structural deficiency of electrons (p volume), permitting the conduction of electrons. With the creation of mobile charges by light absorption there follows a flow away from the chlorophyll aggregate and across the semiconducting layers. If the potential between these two layers could be measured, it would be found equivalent to ~40 kcal/mole minus the 5 kcal/mole loss postulated above, or 1·6 V. If the circuit between these two "electrodes" is completed by chemical reactions, as it is in photosynthesis, the potential at each electrode, relative to the ground state of the system, is simply that required by the oxidants or reductants with which the electrons and positive charges must react. Thus, in terms of our arbitrary "ground", which is the standard hydrogen electrode potential, the 1·6 V potential across the "battery" is distributed as 1·3 V positive and 0·3 V negative, since these are the potentials, relative to our "ground", with which the electrodes must react. Viewed in this way, it appears that the recently developed solar battery (CHAPIN *et al.*, 1954) may have been preceded by a similar but much more efficient process in photosynthetic organisms!

The evolution of oxygen from hydrogen peroxide according to the reaction

$$H_2O_2 = \tfrac{1}{2}\, O_2 + H_2O$$

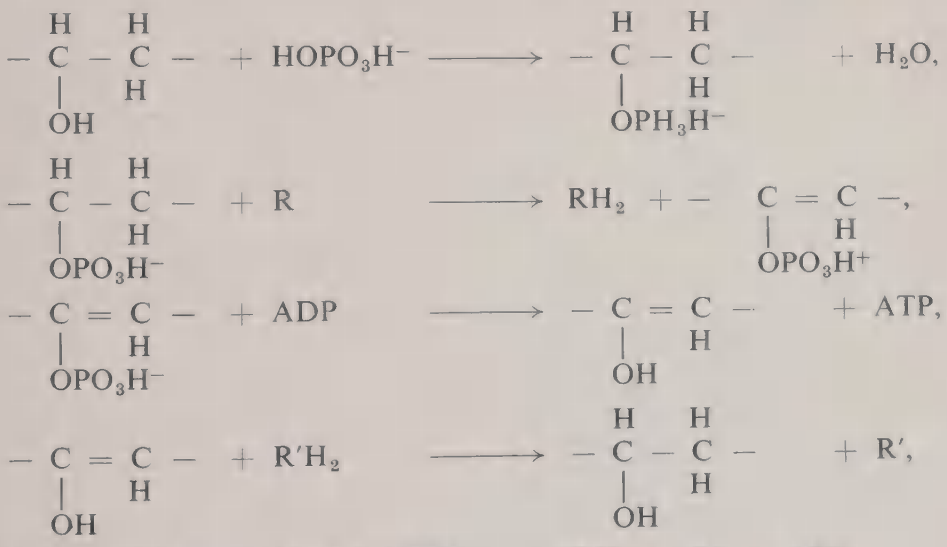
proceeds with a positive potential of 0·37 V for 10^{-6} M peroxide, so that another 8·5 kcal/mole of excess energy is expended per electron. This reaction can be catalyzed by a number of simple inorganic compounds. Inhibition of oxygen evolution in the Hill reaction by orthophenanthroline has been observed (GAFFRON, 1937) so some iron-containing enzyme may be involved. The recent work of CHANCE (1954) has demonstrated the formation of catalase H_2O_2 complexes under aerobic conditions. It appears that this complex can form with concentrations of H_2O_2 as low as 10^{-8} M.

It is attractive to suppose that the peroxide may be used in part to oxidize some ferrocytochrome (CHANCE, 1951) in a reaction catalyzed perhaps by peroxidase. Thus the requirement for an oxidizing agent to react ultimately with the primary reducing agent could be met without requiring oxygen. This is rather useful because of the evidence that molecular oxygen is not required for photosynthesis. ALLEN (1953) has reduced the concentration of molecular oxygen in contact with photosynthesizing organisms to a value of 0·004 mm Hg and finds, at this level, no diminution of the rate of photosynthesis. The absence of a back reaction involving molecular oxygen was also shown by the studies of Brown (1953) with the mass spectrometer and isotopically labelled oxygen.

It is interesting to suppose that the ferrocytochrome oxidized by H_2O_2 is reduced cytochrome f, discovered by Hill & Scarisbrick (1951). It was found that this compound was present in considerable amounts in green chloroplast material and that it had an oxidation-reduction potential of + 0·365 V, about 0·1 V better as an oxidizing agent than cytochrome c. Since the oxidizing potential of H_2O_2 for the physiological conditions as given above is + 1·2 V, there is a potential difference between these two half reactions of 0·8 V, or a negative free-energy change of 37 kcal/mole for the oxidation of two reduced cytochrome f molecules with one H_2O_2 molecule.

The oxidized cytochrome could then react with other electron carriers, perhaps other cytochromes, and eventually with the primary reducing agent or with TPNH. Since the oxidation-reduction potential of the latter is now considered to be —0·324 V (Burton & Wilson, 1953) there is a potential difference between cytochrome and TPN^+ of 0·65 V, or a negative free energy of 30·3 kcal for the oxidation of one mole of TPNH by oxidized cytochrome f.

Very little is known about the mechanism of formation of ATP during the oxidation of TPNH. There is enough energy available in the steps suggested above to bring about the formation of four moles of ATP. One general type of mechanism for the oxidative formation of ATP may involve esterification of a hydroxyl group with inorganic phosphate, dehydrogenation of an adjacent C-C or C-N bond with a suitable oxidizing agent to form a "high-energy phosphate group," a reaction with ADP to form ATP and an unsaturated alcohol, and finally reduction of the unsaturated alcohol to the original substance.



$$\begin{array}{ccc} H & & H \\ -C & - & C- \\ | & & H \\ OH & & \end{array} + HOPO_3H^- \longrightarrow \begin{array}{ccc} H & & H \\ -C & - & C- \\ | & & H \\ OPH_3H^- & & \end{array} + H_2O,$$

$$\begin{array}{ccc} H & & H \\ -C & - & C- \\ | & & H \\ OPO_3H^- & & \end{array} + R \longrightarrow RH_2 + - \begin{array}{ccc} C & = & C-, \\ | & & H \\ OPO_3H^+ & & \end{array}$$

$$\begin{array}{ccc} -C & = & C- \\ | & & H \\ OPO_3H^- & & \end{array} + ADP \longrightarrow \begin{array}{ccc} -C & = & C- \\ | & & H \\ OH & & \end{array} + ATP,$$

$$\begin{array}{ccc} -C & = & C- \\ | & & H \\ OH & & \end{array} + R'H_2 \longrightarrow \begin{array}{ccc} H & & H \\ -C & - & C- \\ | & & H \\ OH & & \end{array} + R',$$

$$R + R'H_2 + ADP + H_2PO_4^- \longrightarrow RH_2 + R' - ATP.$$

The $-CH_2-$ group of the reacting alcohol can also be $-NH-$. Considering the number of steps involved in the above mechanism, we could expect that at least 5 calories negative free-energy change would be required for the entire process, so that about 15 kcal/mole might be required for the formation of ATP by such a mechanism. It is thus possible that anywhere from 1 to 4 molecules of ATP might be formed for each molecule of primary reducing agent used, provided electron carriers of suitable intermediate potentials are available. What these may be is difficult to say, but some cytochromes, for example cytochrome b, lie intermediate between cytochrome f and TPNH.

The process postulated for the formation of primary reductant and H_2O_2 has a quantum requirement of four for each four electrons transferred or two for each RH_2 formed. The overall quantum requirement depends on the number of RH_2 molecules that must be used to form ATP. If all required ATP can be supplied from respiration reactions outside the chloroplast, as may be the case at very low light intensities, the overall quantum requirement is four. At high light intensities the over-all quantum requirement will be 10, 7, 6, or 5 depending on whether the number of ATP molecules formed per RH_2 burned is 1, 2, 3, or 4 respectively.

The controversy regarding the minimal requirement of photosynthesis has not been settled. The recent experiments reported by WARBURG *et al.* (1953) are very convincing, since quantum yields of four and even three are reported with high light intensities for long periods of time and without corrections for respiration, thus effectively answering criticism based on the possibility that the reported quantum requirements are due to contribution of respiratory energy to photosynthesis.

This leaves only criticisms based on the evaluation of the manometric technique. No such evaluation is attempted here, but it may be worthwhile to point out one possible difficulty. Calculations of oxygen evolution and carbon dioxide uptake by the two-vessel method depend on the assumption of constant solubilities of these gases. However, the solubility of carbon dioxide may change significantly if the pH of the medium changes, and this in turn could be influenced by the secretion of acid by the algae. For example, it has been observed in this laboratory that in high light intensities algae produce glycolic acid. TOLBERT (NATHAN E. TOLBERT, private communication) has found that glycolic acid formed in strong light by algae is secreted into the medium.

In view of this and many other difficulties inherent in the manometric determination of quantum yields, it has seemed desirable to try other methods of measuring oxygen liberation for quantum-requirement calculations. One such study is that of BRACKETT *et al.* (1953) who used a polarographic determination of oxygen and calculated a quantum requirement as low as six. A relatively simple and straightforward experiment has now been carried out using an oxygen analyser employing paramagnetic measurement of oxygen (BASSHAM *et al.*, 1955).

The measurements of photosynthetic rate were dependent only on the measured change in the percentage of oxygen in a closed gas-circulating system and the known volume of the system. Both of these are directly measured quantities which were checked frequently with standard gas mixtures.

The algae, *Chlorella pyrenoidosa*, were grown according to previously described conditions (BENSON *et al.*, 1949) in 4% CO_2. The quantum requirements of these algae were tested after a variety of preconditions. The best condition found was selected and determinations were made as a function of light intensity. The values of the quantum requirement were determined both for the uncorrected rate of photosynthesis and for the rate, which we will call photosynthesis, obtained by subtracting the dark-respiration rate from the uncorrected rate.

This correction seems justified in view of BROWN's study (1953) in which isotopic oxygen was used to demonstrate that no significant change in respiration of *Chlorella pyrenoidosa* rate took place during alternate fifteen- or twenty-minute periods of light and dark. The same paper, as well as the earlier ones by EMERSON & LEWIS (1943), WEIGL *et al.* (1951) and BRACKETT *et al.* (1953), showed an increase in the dark-respiration rate (and the light rate as well in BROWN's paper) that is produced by conditioning the plants with photosynthesis instead of leaving them in the dark for several hours. This indicates a photosynthesis enhancement of the rate of respiration over the rate required by the plants while resting in the dark.

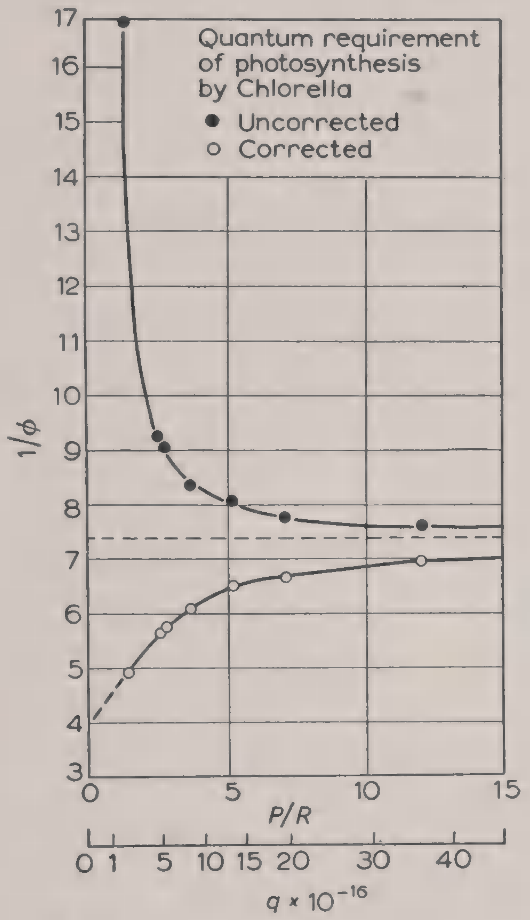


Fig. 3. Relation between quantum requirement in photosynthesis and the ratio of photosynthesis to respiration rate

If each of the experimentally determined quantum requirements is plotted against the ratio of photosynthesis to respiration (p/r), an interesting result is obtained. At high ratios of p/r, where it would be expected that respiration could contribute relatively little of the ATP required for photosynthesis, both corrected and uncorrected quantum requirements approach the same value, about 7·4. At low values of *p*/*r*, where respiration could contribute relatively more ATP, the corrected quantum requirement approaches four while the uncorrected quantum requirement becomes very great. This result, shown in Fig. 3, lends credence to the theory that the two molecules of primary reductant required for the reduction of one molecule of CO_2 are generated by 4 quanta

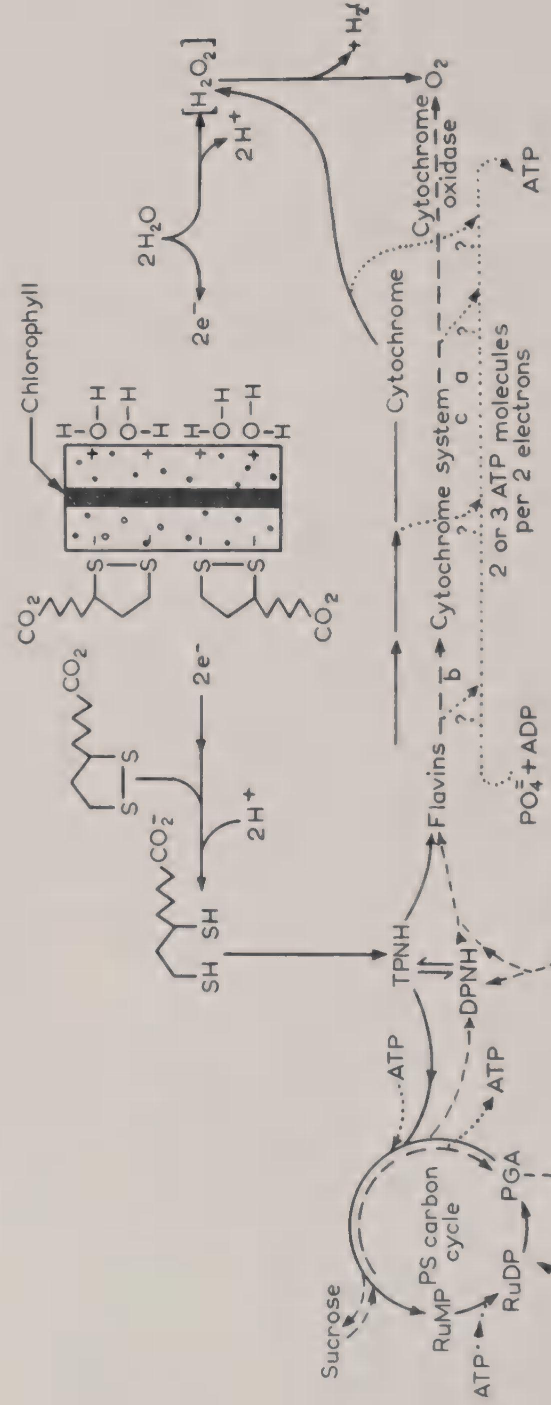


Fig. 4. Relations of carbon, phosphorus and electron flow in photosynthesis and respiration.

but that when the ATP required for the reduction of CO_2 must be formed by reactions consuming the reducing agent, there is a net requirement of about 6 or 7 quanta for each CO_2 molecule reduced.

The relationships of energy transfer in respiration and in photosynthesis are shown in Fig. 4. It will be seen that in this scheme the principal "innovations" required for photosynthesis as compared with respiration are the photochemical "battery" and the use of perhaps two specialized electron carriers, thioctic acid and cytochrome f. As more is known about details of the energy-transferring processes, we may expect that additional special steps will be found.

Biosynthesis of Chlorophyll

Beyond the obvious importance of chlorophyll as the most important pigment involved in absorption of electronic energy and its conversion to chemical energy, there appears at present no relation between the biosynthesis of chlorophyll and the mechanism of photosynthesis. Perhaps other relations will be found when more is known about the early photochemical processes. In any event, the pathway by which chlorophyll is synthesized in the plant from CO_2 and nitrate or ammonia is interesting in itself and has been the subject of a long series of experiments by GRANICK (1951) and SHEMIN (1952) who have worked out the detailed biosynthetic sequence.

At the International Conference on the Peaceful Uses of Atomic Energy in 1955, GODNEV reported a series of experiments (GODNEV & SHLIK, 1955) in which the biosynthesis of chlorophyll was studied by feeding plants $C^{14}O_2$ and C^{14}—labelled acetate. The leaves of higher plants were shown to incorporate both of these substances into chlorophyll. A nineteen day half-life for the chlorophyll in the mature tobacco leaf was calculated but this calculation was based on a single twenty-four hour point on the incorporation curve and is consequently subject to some question.

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II. SYNTHESIS AND METABOLISM OF PLANT PRODUCTS

Abstract—Some examples of the numerous experiments in which C^{14} has been used for biosynthesis of labelled plant products, and studies of plant metabolism are reported.

The types of experiment included are:

1. Techniques for the biosynthesis of uniformly labelled plant products by exposing seedlings to $C^{14}O_2$, and the use of detached leaves for synthesis of sugars and starch.
2. Synthesis of sucrose and maltose in which one hexose moiety only is labelled, by enzymic transfer reactions.
3. Studies of carbohydrate metabolism in which labelled sugars are supplied to leaf tissue and the redistribution of labelling determined.
4. The use of glucose labelled in carbon atoms 1 or 6 in investigations of the synthesis of cellulose and other polysaccharides.
5. Experiments on translocation in plants.
6. Enzyme mechanisms for hexose transfer.

The advent of C^{14} has made possible renewed investigation not only of the chemistry of photosynthesis but of other outstanding problems in the biochemistry of higher plants.

It is perhaps not surprising that considerable attention has been paid to aspects of carbohydrate metabolism, but at the same time information on a wide variety of topics involving a large number of different plant products has accumulated as a result of tracer studies using C^{14}.

The usefulness of the higher plant for the biosynthesis of natural products uniformly labelled with C^{14} was early recognised, and many preparations of radioactive plant products ranging from glucose to complex alkaloids have been carried out. By introducing such preparations into plant tissues and determining the redistribution of radioactivity within the tissue great progress has been made in the understanding of metabolic processes and pathways of synthesis. More recently metabolic studies have been carried out using substrates, chemically synthesised, in which one or more known carbon atoms of the molecule only are radioactive. Movement of materials within the plant and the nature of the translocated material have also been followed by tracer methods, and finally labelled substrates have been employed in elucidating the mechanism of enzyme action *in vitro* especially in the study of transfer reactions.

Some examples of the types of study outlined above are discussed in this review, but it is stressed that no attempt has been made to mention all the published work.

SYNTHESIS OF PLANT PRODUCTS UNIFORMLY LABELLED WITH C^{14}

(*a*) *By Exposing Whole Plants to* $C^{14}O_2$

It is obvious that if seedlings are allowed to assimilate $C^{14}O_2$ as the plant grows all its constituents will become uniformly labelled with C^{14}. SCULLY and his colleagues at the Argonne National Laboratory have studied in detail the requirements which must be met for this process of uniform labelling to be carried out efficiently. In his report at the Geneva Conference (SCULLY *et al.*, 1955), SCULLY described the design and construction of biosynthesis chambers for growing plants on a rather large scale in $C^{14}O_2$, and discussed such factors as height and weight of plant, length of culture period, concentration of $C^{14}O_2$, temperature, humidity, daylength, light intensity and mineral nutrition. As an example of the use of the chambers an abridged account of the culture of tobacco plants and the study of tobacco flavanoids is reproduced below. It is pointed out that in experiments of this type the presence of compounds which have hitherto escaped identification because of their low concentration may be revealed since the carbon-14 tag allows detection of amounts about 10 times less than can be achieved by the usual chemical methods.

Seed of *Nicotiana rustica* was planted in April in soil in the greenhouse and maintained on 18-hour daylength. Six weeks later the soil was carefully washed from the seedling roots which were then transplanted into fine quartz sand contained in two-quart glazed crocks having bottom-drains. At this time the plants were supplied a complete nutrient solution. Three weeks later eight of the more vigorous seedling plants, which had four small, partially expanded leaves, were carefully transplanted to quartz gravel in the C^{14} biosynthesis chamber.

The complete nutrient solution was supplied twice daily to plants. The temperature and humidity, respectively, were maintained at $75° \pm 2°$ F and 50–55% relative humidity. Two days later the chamber was hermetically sealed and the atmosphere was charged with $C^{12}O_2$ to raise the concentration of CO_2 to 0·060%; at the same time enough $C^{14}O_2$ was released to adjust the specific activity of the air phase to 350 μc/g C. After 4 hours of photosynthesis the plants had dropped the CO_2 concentration to 0·052% and the specific activity had dropped to 300 μc/g C. The following morning the CO_2 concentration had risen to 0·062% while the specific activity of the carbon-14 in the air phase had dropped to 200 μc/g C. These figures reflect the usual dilution effect which plant respiration has upon the specific activity of the atmosphere early in a biosynthesis period. As the biosynthesis period progressed the plants became more uniformly labelled and the specific activity of the air phase was progressively less influenced by respiratory CO_2. Essentially the plants became uniformly labelled at the desired specific activity when it was observed that the activity of the air phase remained constant throughout a dark period.

At intervals, usually at the start of a photosynthesis period, a new charge was introduced into the chamber to raise the CO_2 concentration and, if necessary, to readjust the specific activity to the desired level. It was the intent of this experiment to tag the entire plant at a specific activity of 250 μc/g C. Therefore in the early stages of the biosynthesis the specific activity of the air phase was adjusted to 350 μc/g C, after two weeks to 200 μc/g C, and in a few more days to 275 μc/g C. Throughout the entire experimental period, the CO_2 level was maintained within the concentration range of 0·100% to 0·010%. When the plants lowered the atmospheric CO_2 concentration to 0·010%, a new charge was introduced to raise the CO_2 to the maximum level and adjust the specific activity.

Forty-seven mc were fixed by the plants and about 3·8 kilograms of fresh plant tissue were produced. Four of the eight plants were harvested on the twentieth culture day, two more on the thirty-first day and the final two on the forty-fourth or last day. The plants were divided into leaves, stems, roots and flowers at harvest time and stored either in a deep freeze or in 85% isopropyl alcohol.

The leaf tissue was transferred to boiling 85% isopropyl alcohol and boiled for 5 minutes to destroy enzymatic activity. The tissue was then disintegrated in a Waring blendor, transferred quantitatively to a Soxhlet extractor and extracted for 16 hours with 85% isopropyl alcohol. The alcohol was evaporated from this solution. The fats, pigments, tannins and other water insolubles were removed by filtration, and the flavanoids further purified by extracting with isoamyl alcohol. The isoamyl alcohol was evaporated to dryness under reduced pressure in the presence of nitrogen. The residue was taken up in a minimum of 80% ethyl alcohol for paper chromatographic analysis. Since most of the flavanoids and other polyhydroxy phenolic compounds exhibit fluorescences in ultra violet light, these compounds can be seen by examination under the ultra violet lamp provided their concentration is of the order of 5–10 micrograms. By this means and by the use of appropriate sprays, six spots of compounds were evident. A radio-autograph revealed 20 spots.

Previous to this work three flavanoid compounds had been reported in tobacco leaves, namely, rutin, isoquercitrin and quercetin. By the use of the above technique at least four additional flavanoids previously unreported have been shown to occur naturally in tobacco leaves. In order that these compounds be further identified and characterised by classical chemical procedures, much larger quantities than normally employed with paper chromatographic methods have to be processed. Such large-scale separation procedures can be worked out using small amounts of tagged compounds.

A further interesting use of the Argonne facilities has been a study of rubber formation and turnover in *Hevea braziliensis*. These studies have determined the rate at which current assimilate is translocated from the leaf to other organ sites, as well as the rate of conversion of assimilate into rubber in latex in the different organs. Using intact young plants it was found that tagged rubber first appeared in leaf latex, suggestive of the possibility that the precursors of the rubber molecule are predominantly formed in and supplied from this organ. Trees approximately 3–4 inches in diameter were tapped for latex 3 times a week during the course of a 115-day C^{14} culture period. The results of assay of latex solvent fractions showed that a marked correlation existed between the rate at which C^{14} appeared in latex constituents soluble in alcohol and that at which it appeared in isolated rubber.

Other *Hevea* trees containing rubber tagged at a given concentration of C^{14} exhibited approximately a 10-fold loss of rubber activity when defoliated and retained in normal atmospheres. Since no appreciable variation in total rubber concentration occurred in this experimental period the data are taken to indicate that rubber, once formed, does turnover at a measurable rate, a point which has been in question for some time (cf. KURSANOV, 1955).

Consideration has also been given to effects of radiation upon growth. As much as a 10-fold difference in species sensitivity to radio carbon has been observed. Red kidney bean grown in atmospheres of $C^{14}O_2$ with specific activities up to 2 000 μc per gramme of carbon showed deformation and chlorosis of leaves at 1 000 μc and above; and as well necrosis of the apical meristems. In other experiments it was found that seeds, harvested from plants apparently unaffected by exposure to radiation, yielded seedlings whose growth was markedly arrested in comparison with those from seeds of plants not exposed to radiation.

Using closed assimilation chambers in a similar manner to that employed at Argonne, GEILING and his colleagues have prepared a number of alkaloids and other drugs. For example colchicine was prepared from corms of *Colchicin autumnale* (WALASZEK *et al.*, 1949). by exposing plants every third day over a period of 20 to 30 days to doses of $C^{14}O_2$ so that each plant received 300 to 500 μc. The isolated colchicine had a specific activity of 27 μc/g, and five other radioactive alkaloids were detected. Digitoxin was prepared from the air dried leaves of *Digitalis purpurea* (OKITA *et al.*, 1954) after exposure for 4 to 6 weeks to $C^{14}O_2$ in five divided doses giving 4·2 mc per plant. The specific activity was 0·26–0·54 μc/mg. Preparations of radioactive morphine (GEILING *et al.*, 1954) and nicotine (GANZ *et al.*, 1951) have also been reported.

Another interesting preparation is that of galactopyranosyl glycerol from the marine red alga *Irideae laminaroides* (BEAN, PUTMAN & HASSID, 1953). The thallus was exposed to $C^{14}O_2$ in the light and the galactoside isolated, hydrolysed and the products separated. Approximately 25% and 12% of the activity of the $C^{14}O_2$ was incorporated into galactose and glycerol respectively.

(*b*) *By Exposing Detached Leaves to $C^{14}O_2$*

Preparations of starch and sugars are more conveniently made using detached leaves, since a large proportion of the CO_2 assimilated then appears as carbohydrate. Tobacco leaves which are good starch formers have been frequently used, and canna leaves which rarely form starch have been used particularly for the preparation of sucrose. Two examples are described. PUTMAN & HASSID (1948) prepared radioactive starch and sugars by exposing a single tobacco leaf, depleted of carbohydrate by keeping the plant in the dark, to $C^{14}O_2$. $C^{14}O_2$ was released into the leaf chamber by the action of lactic acid on about 550 mg $BaC^{14}O_3$, giving an initial concentration of $C^{14}O_2$ of about 10%. After 18 to 24 hours illumination with two 100-watt lamps most of the $C^{14}O_2$ had been assimilated. Sugars were then extracted in hot 80% ethanol and fructose and glucose isolated using carrier. Starch was extracted from the thanol insoluble material with $CaCl_2$ and purified by means of its iodine complex. Sucrose was prepared from the ethanol extract of canna leaf. SCULLY (*loc. cit.*) quotes a similar experiment using canna leaf in which sucrose containing 175 mc was isolated after 5 hours assimilation; 57% of the isotopic C incorporated being recovered as sucrose.

The method of PUTMAN & HASSID was adapted by PORTER & MARTIN (1952) for the preparation of larger amounts of material. A shallow Perspex leaf chamber holding up to 12 tobacco leaves was connected in a closed circuit with a flask in which $C^{14}O_2$ was generated from 15 g of $BaC^{14}O_3$, and a pump which circulated the gas continuously over the leaf surface. Carbon dioxide was generated in two doses and in bright sunlight (5 000–8 000 ft candles), 14 to 18 sq.dm of leaf surface assimilated CO_2 equivalent to 2·25 g of hexose, by which time concentration of CO_2 had fallen from 10% to 0·02%. 60 to 80% of the carbon assimilated was recovered as starch and sugar, with a specific activity per milliatom of C about 15% less than that of the $C^{14}O_2$. The leaves were macerated in a Waring Blendor and starch isolated by centrifugation. Fructose and glucose were isolated from the aqueous extract by passage through a charcoal column and separated from one another on a Florex. xxx. column.

The distribution of labelling after long periods of photosynthesis was investigated by GIBBS (1949). Invert sugar isolated from leaves after 24 hours in light was degraded by *Lactobacillus casei*, and it was found that activity was equally distributed between the pairs of carbon atoms of the hexose chain 3,4; 2,5 and 1,6. ABRAHAM, PUTMAN & HASSID (1952) applying chemical methods of degradation to glucose extended the findings by demonstrating equality of labelling in all 6 carbon atoms. Similar results were obtained by VITTORIO, KROTKOV & REED (1950).

Detached leaves have also been used for synthesis of radioactive amides. WILSON *et al.* (1951) used lupin seedlings for synthesis of asparagine. 8 day old seedlings were cut off and the stems placed in 0·1% ammonium chloride, and exposed to 5% $C^{14}O_2$ for 3 days. BIDWELL *et al.* (1954) examined leaves of beet, swiss chard and tobacco as possible sources of glutamine and obtained the best yields with swiss chard. When swiss chard leaves were placed with their petioles in 0·1% ammonium chloride and exposed to $C^{14}O_2$ glutamine continued to increase for 5 days and in one experiment then amounted to 5% of the fresh weight. The activity incorporated into glutamine was too high to be accounted for only by new synthesis and some turnover is suggested. 28% of the C^{14} assimilated was recovered in 96·5 mg of isolated and purified glutamine.

ENZYME SYNTHESES OF SUCROSE AND MALTOSE WITH ONE HEXOSE MOIETY LABELLED

The importance of sucrose in the metabolism of plants has stimulated interest in syntheses of sucrose in which only one hexose moiety is uniformly labelled. Wolochow *et al.* (1949) used the sucrose phosphorylase of *P. saccharophila* which effected transfer of glucose from sucrose to radioactive fructose, so yielding sucrose labelled in the fructose moiety. Using the same enzyme, sucrose labelled in the glucose moiety was prepared by transfer of glucose from labelled glucose-l-phosphate to inactive fructose. The glucose-l-phosphate was prepared by the action of potato phosphorylase upon radioactive starch. The sucrose samples were shown to contain less than 1% of the total activity in the hexose moiety expected to be inactive. Porter & May (1955) repeated this synthesis of sucrose labelled in the fructose moiety, but for synthesis of sucrose labelled in the glucose moiety they used the invertase of *Aspergillus oryzae.* This enzyme will transfer fructose from sucrose to glucose, so that using inactive sucrose and active glucose the required labelling is achieved. Under the conditions used about 10% of the activity of the glucose became incorporated in sucrose (see also pp. 148 and 151).

In a similar manner maltose in which either the reducing or non-reducing glucose moieties are labelled has been prepared using the phosphorylase of *Neisseria meningitidis* (Fitting & Putman, 1952), which in a reversible reaction acts upon glucose-l-phosphate and glucose to give maltose. Maltose uniformly labelled was prepared by α-amylolysis of radioactive starch and used for preparation of active glucose-l-phosphate.

These reactions for the synthesis of sucrose and maltose in which one hexose moiety only is labelled are set out below, using the symbols G O H and F O H to represent glucose and fructose and * to denote radioactivity.

for sucrose labelled in the fructose moiety:

$$G - O - F + F^* - O - H \underset{\text{phosphorylase}}{\overset{\text{sucrose}}{\rightleftarrows}} \underset{\text{sucrose}}{\underline{G - O - F^*}} + F\,OH$$

for sucrose labelled in the glucose moiety:

$$\text{I.}\ \underset{\text{starch}}{(G^*OH)_n - (n-1)\,H_2O} + xH_3PO_4 \underset{\text{phosphorylase}}{\overset{\text{potato}}{\rightleftarrows}} (G^*OH)_{n-x}$$

$$- (n - 1 - x)\,H_2O + x(G^* - O - PO_3H_2)$$

$$G^* - O - PO_3H_2 + FOH \underset{\text{phosphorylase}}{\overset{\text{sucrose}}{\rightleftarrows}} \underset{\text{sucrose}}{\underline{G^* - O - F}} + H_3PO_4$$

$$\text{II.}\ G - O - F + G^* \underset{\text{invertase}}{\overset{\text{A. oryzae}}{\rightleftarrows}} \underset{\text{sucrose}}{\underline{G^* - O - F}} + G$$

for maltose labelled in the reducing moiety:

$$G - O - PO_3H_2 + G^*OH \underset{\text{phosphorylase}}{\overset{\text{maltose}}{\rightleftarrows}} \underset{\text{maltose}}{\underline{G - O - G^*}} + H_3PO_4$$

for maltose labelled in the non-reducing moiety:

$$\underset{\text{starch}}{(G^*OH)_n - (n-1)H_2O} + H_2O \xrightleftharpoons{\alpha\text{ amylase}} G^* - O - G^* + \text{dextrins}$$

$$G^* - O - G^* + H_3PO_4 \xrightleftharpoons[\text{phosphorylase}]{\text{maltose}} G^* - O - PO_3H_2 + G^*OH$$

$$G^* - O - PO_3H_2 + GOH \xrightleftharpoons[\text{phosphorylase}]{\text{maltose}} \underset{\text{maltose}}{\underline{G^* - O - G}} + H_3PO_4$$

FRENCH *et al.* (1953) have reported that the amylase of *B. macerans* will act upon cyclohexa-amylose in the presence of glucose and distribute the glucose among a series of linear polymers of glucose, including maltose. This reaction has provided another method of preparing maltose labelled in the reducing moiety and at the same time low molecular weight dextrins labelled in the terminal reducing glucose unit. The reaction products may be separated on paper or by gradient elution from charcoal with ethanol. The isolated maltose has been converted to maltobionic acid (FITTING & PUTMAN, 1952), to maltose-l-phenylflavazole (FRENCH *et al.*, *loc. cit.*) or to maltitol (PORTER & REYNOLDS, unpublished). The hydrolysis products of these three maltose derivatives were separated by paper chromatography, and in this way it was shown that activity was localised in the fraction corresponding to the reducing glucose unit of the maltose. Presumably cyclohexaamylose could be prepared uniformly labelled throughout by the action of *B. macerans* on radioactive starch. If the isolated product was then used in the reaction already discussed but with inactive glucose the maltose formed should be labelled only in the non-reducing glucose moiety. This synthesis has not yet been described.

LELOIR & CARDINI (1953) demonstrated synthesis of sucrose *in vitro* from uridine diphospho-glucose (UDPG) and either fructose or fructose-6-phosphate using enzymes from wheat germ and other plant tissues. BURMA & MORTIMER (in press) have now investigated this system in sugar beet leaves and leaf homogenates. When radioactive glucose-l-phosphate (G—l—P) was supplied to a leaf, sucrose was synthesised in which both moieties were active (see also p. 141). When uridine triphosphate (U.T.P.) was supplied as well, the yield of sucrose was reduced and a relatively large proportion of the radioactive glucose was converted to UDPG. Leaf homogenates converted G—l—P to active glucose and fructose and also yielded UDPG from G—l—P and U.T.P. Sucrose labelled in the glucose moiety was synthesised by the sequence of reactions shown below using beet leaf homogenates.

$$UTP + G^* - 1 - P \longrightarrow UDPG^* + P - P$$

$$UDPG^* + F - 6 - P \longrightarrow \underset{\text{sucrose}}{(G^* - O - F)P} + UDP \longrightarrow G^* - O - F + P$$

$$UDP + ATP \longrightarrow UTP + ADP$$

UTILIZATION OF LABELLED SUGARS IN METABOLIC STUDIES

Metabolism of labelled sugars introduced into plant tissues, usually leaves, has been studied in a number of laboratories. Sugars were introduced by vacuum infiltration of small amounts of highly active material which did not sensibly alter the amounts already present, or by floating leaf disks or placing leaf petioles in solutions of radioactive sugars, in which case relatively large amounts of sugar enter the tissue. PUTMAN & HASSID (1954) in a study of sucrose synthesis infiltrated uniformly labelled glucose or fructose into canna leaf disks and examined the redistribution of activity and evolution of CO_2 at intervals during a period of 6 hours in darkness. Sucrose was synthesised from both hexoses and shown to be strongly labelled in both moieties. Little or no activity was found in the free hexose not supplied as substrate. Paper chromatography of ethanol extracts of the leaf disks followed by radio autography revealed the presence of active sugar phosphates immediately following infiltration but this activity disappeared with time and at the end of six hours most of the remaining activity was in sucrose.

Infiltration of uniformly labelled sucrose or sucrose in which only the fructose moiety was labelled showed that there was considerable inversion of sucrose immediately on entry, followed by some resynthesis. The newly formed sucrose was strongly labelled in both moieties even when sucrose labelled only in the fructose moiety was infiltrated, but free glucose remained inactive. The authors concluded that free hexose is not involved in sucrose synthesis, which probably proceeds from phosphorylated hexoses, and that since free hexose did not become labelled the site of sucrose synthesis must be distinct from that of inversion. PUTMAN & HASSID suggested that in their experiments the inversion was extra-cellular (but see below).

Further points noted were that sugars accounted for 80% of the activity immediately after infiltration although there was a rapid incorporation of some activity into the ethanol insoluble fraction of the leaf tissue, more especially when glucose was infiltrated. After 6 hours of the total activity remaining only 52% was in sugar, owing to further redistribution into other compounds. The progress of incorporation of activity in the experiment where glucose was infiltrated is shown in Fig. 5.

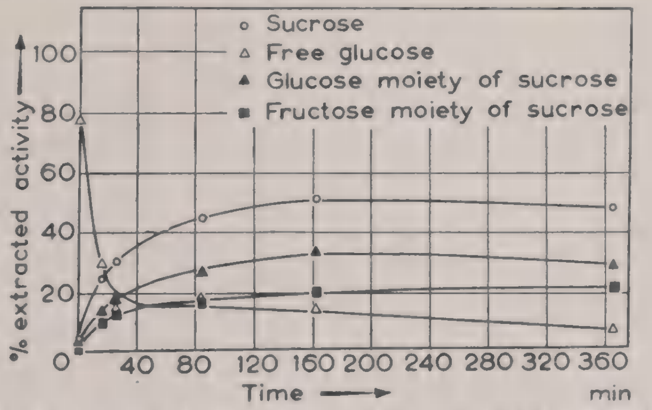

Fig. 5. Distribution of activity in sugars of Canna leaf disks after infiltration of C^{14} labelled glucose—as per cent of activity in the ethanol extract. (PUTMAN & HASSID, 1955.)

PORTER & MAY (1954) studied sugar utilization by tobacco leaf disks, with particular reference to starch synthesis from sugar. Disks depleted of carbohydrate were floated on 5% solutions of invert sugar or sucrose in which the glucose or fructose component was radioactive. By use of the Warburg technique gas exchange over a 24 hour period was followed. After the first few hours specific activity of CO_2 evolved had risen to a value equal or nearly so to that of the sugar supplied. Measurements of the specific activity of sugar and starch accumulating in the disks showed that a steady state was reached when the sugar had penetrated across the disks and the incoming material was then divided among the several components in almost constant proportions. The mean values of specific activity for the 24 hour periods are shown below in Tables 1 and 2 (reproduced from PORTER & EDELMAN, 1955).

Table 1—Radioactivity of Starch Isolated from Tobacco Leaf Disks after Floating for 24 hours on 5% Solutions of Radioactive Sugars, and of the Carbon Dioxide Evolved (Calculated as Hexose)
(after PORTER & MAY, 1955)

	Relative specific activities. c.p.m./10^{-3} mg.		
	Sugar supplied	Starch	Carbon dioxide
Sucrose. (fructose)*	8·0	7·2	7·8
,, (glucose)*	8·3	9·7	9·2
Invert sugar. (fructose)*	7·1	7·9	7·7
,, ,, (glucose)*	11·5	10·2	9·6

Table 2—Radioactivity of Sugars Accumulated in Tobacco Leaf Disks Floated on 5% Solutions of Radioactive Sugars for 24 hours

	Relative specific activities. c.p.m./10^{-3} mg.					
	Sugar supplied	S†	Fm†	Gm†	F_f†	G_f†
Sucrose. (fructose)*	8·0	8·0	22·3	0·9	20·4	1·9
,, (glucose)*	8·3	8·3	2·9	17·8	2·5	18·0
Invert sugar. (fructose)*	7·1	6·9	8·7	5·3	11·0	5·0
,, ,, (glucose)*	11·5	13·3	10·3	11·7	9·8	16·6

* Hexose residue or free hexose uniformly labelled with C^{14}.

† S, sucrose. Fm and Gm, fructose and glucose moieties of sucrose. F_f and G_f, free fructose and glucose.

Table 1 shows that the specific activity of the starch formed and of the carbon dioxide evolved were equal to one another and equal or nearly so to that of the sugar supplied in each case. The conclusion was drawn that starch and carbon dioxide were derived from a common source and arose solely from the sugar supplied. The common source was presumed to consist of derivatives of glucose and fructose which permitted of rapid interconversion of these hexoses, since glucose and fructose whether supplied free or combined in sucrose contributed in equal measure to both starch and carbon dioxide. There could be no question of preferential use of one or other hexose as a precursor of either starch or carbon dioxide. In these experiments relatively large amounts of sugar accumulated in the leaf disks. The specific activities are shown in Table 2.

When sucrose was supplied the sucrose in the disks retained the asymmetry of labelling and at the same time free hexoses which appeared in large amount had the same labelling as the sucrose moieties. They were therefore assumed to arise by inversion and at a site remote from that of starch synthesis since there was no exchange of labelling. When invert sugar was supplied there was synthesis of sucrose as well as starch, in which both hexose moieties were strongly labelled. Free hexoses were in this case strongly but unequally labelled, a circumstance which was thought to arise from mixing of invert sugar in which one hexose only was labelled and the products of inversion of sucrose in which both moieties were labelled, again assuming that inversion took place out of contact with the hexose interconverting system. The experiments further suggested that the link between starch and CO_2 was effected through sugar derivatives, possibly phosphate esters, and that free sugar was not involved.

PORTER & MAY (1955) while in agreement with PUTMAN & HASSID (1954) that sucrose inversion takes place at a different site from hexose interconversion, expressed the view that in their experiments the site of inversion might be the vacuole, that is intra- and not extra-cellular. Their opinion was based on the fact that the excess sucrose accumulating in the tobacco leaf disk had the same labelling as the sucrose supplied. (See Table 2.)

VITTORIO, KROTKOV & REED (1954) using the petiole feeding technique, studied uptake and utilisation of uniformly labelled glucose by tobacco leaves in light. The petioles were placed in $0{\cdot}2M$ glucose, and after six hours the leaves were analysed. There had been marked synthesis of sucrose, which was the most strongly labelled substance present, and again both moieties were strongly active, while free fructose was only weakly active. A feature of these experiments was that very little activity appeared in starch. The authors concluded that the radioactive glucose had not been used in light respiration, assuming that otherwise reassimilation of $C^{14}O_2$ would have yielded radioactive starch. Using whole leaves in this manner however the site of sucrose synthesis is uncertain, and it is not known how much of the entering glucose reached the starch synthesising sites.

Similar experiments were done using labelled glucose-l-phosphate with results qualitatively similar, but with lower amounts of incorporation. It seems likely that the ester was hydrolysed and the observed effects were due to the labelled glucose released.

These three series of experiments carried out under very different conditions have yielded similar results with respect to sucrose synthesis and the labelling of free hexose. In general, therefore, it may be postulated that all or part of the sugar entering the cell is incorporated into a metabolic pool by formation of derivatives of fructose and glucose. Rapid equilibration of the fructose and glucose structures occurs and from this source material is drawn for sucrose or starch synthesis and for respiratory substrate. Calvin and his collaborators (BENSON & CALVIN, 1950) have shown that sugar phosphates arise before free phosphates in photosynthesis, and PUTMAN & HASSID (*loc. cit.*) noted the transient formation of phosphate esters during sugar feeding experiments so that these esters may well be the hexose derivatives undergoing interconversion.

All the results also support the notion that inversion of sucrose takes place at a site remote from that of active metabolism and that free hexoses are not direct precursors of sucrose. Further it is agreed that sucrose is not synthesised in plants by an enzyme of the type of sucrose phosphorylase since this requires participation of free hexose; nor is it likely that starch can be formed from sucrose by an enzyme such as amylosucrase, that is by transfer of the glucose moiety.

In support of these general conclusions early experiments using $C^{14}O_2$ may be quoted. For example, GIBBS (1951) found that if sunflower leaves were exposed to $C^{14}O_2$ for times of 1·5 to 4 mins. sucrose became labelled in both moieties but free hexoses remained inactive. GIBBS also records the labelling of an ethanol insoluble material other than starch before sucrose becomes labelled (see also p. 139). This unidentified early product of synthesis does not appear to have been further investigated and merits attention. VITTORIO *et al.* (1954) followed incorporation of $C^{14}O_2$ into starch and sugar in tobacco leaf disks in the light over a six hour period. The progress of incorporation is shown in Fig. 6. Activity appeared in starch before sucrose and in sucrose before free hexose. After six hours the activities became equal and about the same as that of the $C^{14}O_2$ supplied. The results are in accord with the general conclusions of PORTER & MAY (*loc. cit.*) and suggest formation of starch from a common pool prior to formation of free sugar.

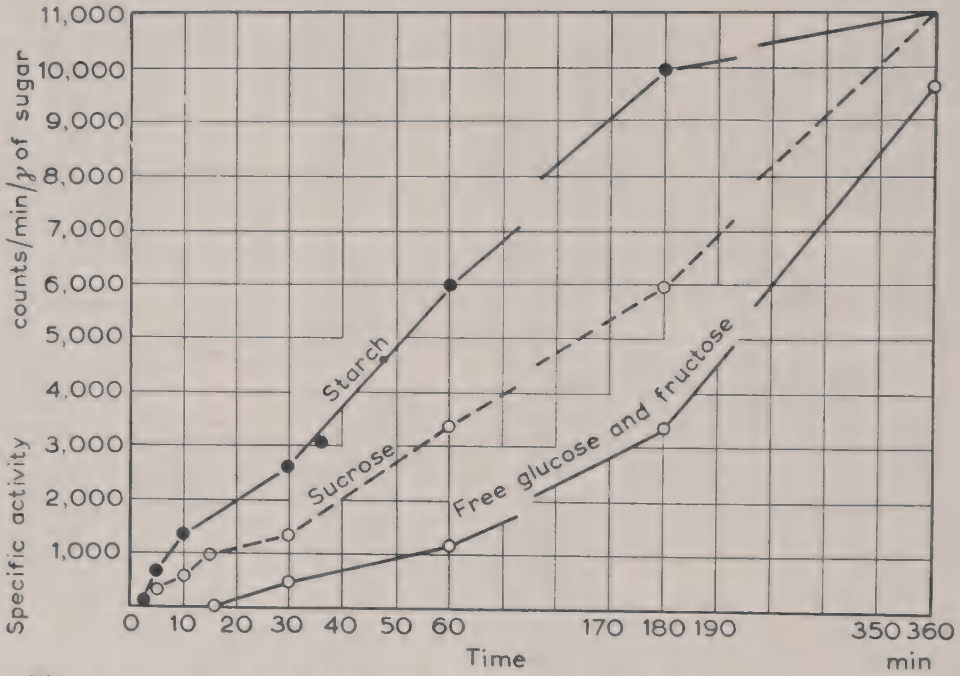

Fig. 6. Specific activities (counts/min) of starch (top curve), sucrose (middle curve), free glucose and fructose (bottom curve) isolated from tobacco leaves after different periods (min.) of photosynthesis of $C^{14}O_2$. (VITTORIO, KROTKOV & REED, 1954.)

Recently BURMA & MORTIMER (in press) have used a somewhat different procedure for studying the fate of assimilated $C^{14}O_2$. Disks of sugar beet leaves were exposed in light for 30 seconds to air enriched with $C^{14}O_2$. The atmosphere was then replaced by normal air and the disks kept in light or dark for an additional 4 minutes. After 30 seconds, ethanol soluble radioactive products included phosphate esters of sugars, pyruvic and glyceric acids (60%), sucrose (15%) and smaller amounts of glycine, serine, malic and other acids. After 4 minutes in light without $C^{14}O_2$ 80% of the activity soluble in ethanol was in sucrose. After 4 minutes in dark however activity in sucrose increased only slightly, but increase was renewed if light was readmitted.

There were also differences in light and dark in the change in activity of glycine and serine as compared with alanine and threonine. The authors suggest that sucrose synthesis requires light energy at some stage beyond the primary assimilation reaction and that the pathways of utilisation of glycine and serine differ from those of alanine and threonine.

The numerous observations in the older literature showing that starch formation from sugar in the dark requires the presence of oxygen might perhaps now be explained as a requirement for ATP to effect phosphorylation of the entering sugar. In an attempt to test this hypothesis RUNECKLES & PORTER (1955) tested the effect of 2·4 dinitrophenol (DNP) on the metabolism of sugars by tobacco leaf disks, reasoning that, in the dark, uncoupling phosphorylation with DNP should inhibit both starch synthesis and respiration. When uniformly labelled sucrose was supplied to tobacco leaf disks starch formation was progressively inhibited as concentration of DNP increased, but as in many other tissues, carbon dioxide production was increased. The specific activity of the CO_2 evolved was, at low DNP concentrations, equal or nearly so to that of the sucrose, showing that the sucrose taken up formed the respiratory substrate. As the DNP concentration was increased the specific activity of the

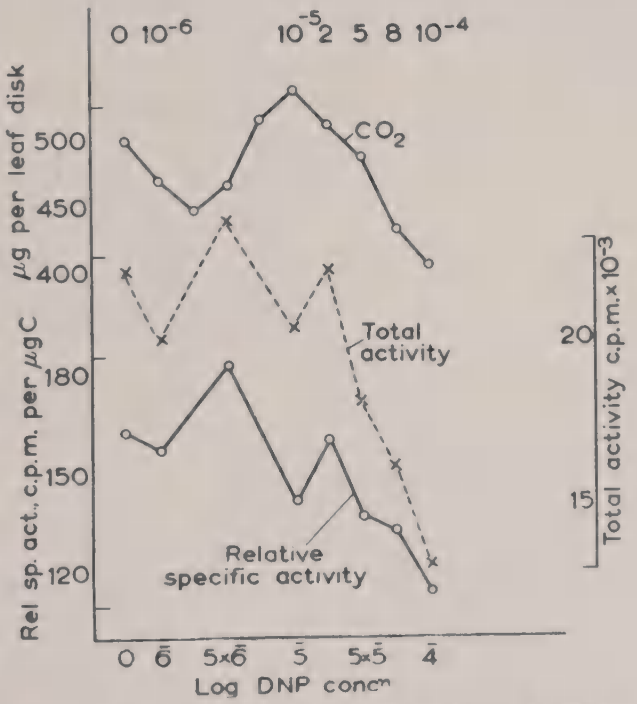

FIG. 7. Output and relative total and specific activities of $C^{14}O_2$ evolved by tobacco leaf disks floated on sucrose uniformly labelled with C^{14}, in presence of 2 : 4 dinitrophenol. (PORTER & RUNECKLES, 1956.)

CO_2 fell. Increasing CO_2 output was therefore accompanied by decreasing use of sucrose as substrate, and increasing use of unlabelled material. DNP thus appeared to restrict the use of sucrose as a precursor both of starch and CO_2 and in this sense the results are consistent with the hypothesis that phosphorylation is the first step in incorporating free sugar into the metabolic pool. They also raise the question as to the nature of the alternative substrate; at present the only information is that respiration quotient fell as sugar utilisation was restricted so that organic acids are unlikely to have undergone increasing oxidation. These effects of DNP are illustrated in Fig. 7.

Kursanov and his colleagues (1955) have made an extensive study of sucrose metabolism in sugar beet, using both $C^{14}O_2$ and uniformly labelled sugars as tracers. The sugars used were isolated from sugar beet leaves after exposure to $C^{14}O_2$. The main purposes of the experiments were to define the site of sucrose synthesis and determine the nature of the material transported to the root. In the course of this work the Russian scientists reached some similar conclusions to those already noted, namely that in 5 minute periods of photosynthesis in $C^{14}O_2$ sucrose was the first free sugar to become radioactive, and that fructose and glucose became active more slowly and to a less degree.

Pavlinova (1954) infiltrated radioactive fructose and glucose into sugar beet leaves on the plant and found that 70% of the hexose was transformed to sucrose. Again in conformity with the results of other workers it was found that sucrose was synthesised if only fructose was infiltrated in which case sucrose was labelled in both moieties but free glucose did not become active (Pavlinova, 1955).

Results of a typical experiment are presented in Table 3.

Table 3—Hexose and Sucrose Content of Sugar Beet Leaves after Infiltration of 0·2M Invert Sugar Uniformly Labelled with C^{14}

(from Pavlinova, 1954)

Time after infiltration	Hexose		Sucrose	
	cpm	mg/g/FW	cpm	mg/g/FW
0	8666	5·77	83	0·05
1 hr.	5858	3·90	1549	1·03
3 hr.	2921	1·95	3935	2·62

(*cpm = counts per minute*).

Radioactive hexoses were also introduced into conducting tissues of the leaf petiole and into pieces of root tissue cut from roots of different ages. Sucrose synthesis was observed in conducting tissues, but in root tissue only weak synthesis was found in young roots and none in older roots at the time of maximum storage of sucrose.

It is of interest that the activity in the hexose introduced into the root tissue disappeared after about 5 hours but was not found in sucrose. The incorporation of activity into sucrose from hexose infiltrated into root tissue of different ages is shown in Table 4.

Table 4—Radioactivity Incorporated into Sucrose from Hexoses Uniformly Labelled with C^{14}, Infiltrated into Root Tissue of Sugar Beet

(PAVLINOVA, 1954)

Date (1953)	Mean wt. per root in g.	Counts introduced in hexose (cpm)	Counts in sucrose after 1 hr.	3 hr.	5 hr.
July 11	9·0	4625	—	1617	1078
,, 22	25·0	4958	354	958	—
August 7	148·0	4563	83	562	687
,, 23	290·0	5250	—	543	335
September 10	—	4086	99	274	164
,, 28	58·0	4500	0	167	94
October 14	70·0	2562	0	42	83

The nature of the translocatory material was investigated by TURKINA (1954), by removing conducting tissues from the bases of petioles of leaves which had been exposed for 5 minutes to $C^{14}O_2$. Most of the activity was in the sucrose of the lamina, but activity was detected in the sucrose of the petiole base, but not in the hexose. When uniformly labelled sugars were infiltrated into leaves greater activity was found in the conducting tissue at the petiole base after an hour when sucrose was introduced than when hexoses were supplied. After 4 hours no activity had reached the petiole parenchyma, showing that movement from the leaf was confined to the vessels. The rate of movement was 70 to 80 cm/hr.

These experiments have established that the main seat of sucrose synthesis was the leaf, and that little synthesis occurred in the root even at the time of maximum sugar storage. They also provided evidence that sucrose was the main sugar transported and was rapidly transferred to the conducting tissues of the leaf. Since leaves contained a high proportion of free hexose these were regarded as secondary products accumulating at a different site. Formation of sucrose in conducting tissues was also demonstrated (PAVLINOVA, 1955) by

Table 5—Incorporation of Radioactivity into Sucrose in Conducting Tissues and Laminae of Sugar Beet Leaves placed with Petioles in a Solution of Invert Sugar Uniformly Labelled with C^{14}

(PAVLINOVA, 1955)

Time after placing in solution	Conducting tissue G.	F.	S.	Leaves G.	F.	S.
	(cpm/g/FW)					
1 hr. m	4714	5430	983	270	957	2185
2 ,,	6570	6920	5000	1327	2139	4594
4 ,,	4621	4667	6507	518	1149	4746

G = glucose. F = fructose. S = sucrose.

placing leaf petioles in 0·5 ml of 10% glucose or fructose. Active sugars appeared in the conducting tissues and after an hour activity was detected in sucrose, and continued to increase when hexose entry had ceased. Some results are presented in Table 5.

The finding that sucrose synthesis did not take place in the sugar beet root led KURSANOV to consider again the question of accumulation against a concentration gradient and to postulate a system of active transport. To test the view that respiratory energy was required to move sucrose from leaf to root, CO was used as an inhibitor of the terminal oxidase system.

Sucrose uniformly labelled with C^{14} was introduced into the veins of two leaves on a single plant. CO was circulated round a length of the petiole of one leaf and air round the other. It was found that activity did not appear below the area exposed to CO but in the absence of CO it appeared not only in the whole length of the petiole but in regions of the root near the petiole base. A diagram of the observed distribution is shown in Fig. 8.

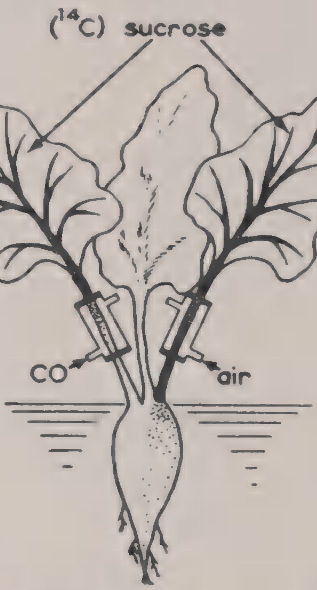

Fig. 8. Diagram illustrating the effect of inhibition of cytochrome oxidase by CO, on the movement of radioactive sucrose in petioles of sugar beet. (TURKINA & DOUBININA, 1954.)

SYNTHESIS OF CELL WALL CONSTITUENTS

Cellulose and Xylan

Work is now developing on the pathway of synthesis of cellulose and related polysaccharides using radioactive sugars labelled in specific carbon atoms usually carbon atoms one or six. GREATHOUSE (1953) presented evidence for direct utilisation of glucose in cotton plants. Glucose labelled in position 1 ($1-C^{14}G$) was introduced into cotton bolls at the time of maximum cellulose synthesis. After 90 days cellulose was isolated and 40% of the activity introduced was found in the glucose derived from it by hydrolysis. This activity was all in the one position which implied direct incorporation of glucose. Later work, however, suggests that this is not the most usual route of synthesis.

Brown & Neish (1954), Edelman, Ginsburg & Hassid (1955) and Shafidzeh & Wolfram (1955) have studied cellulose synthesis in wheat, and all report a greater distribution of activity within the carbon atoms of glucose derived from cellulose, when $1-C^{14}G$ or $6-C^{14}G$ were supplied as substrates than that found by Greathouse.

Brown & Neish injected $1-C^{14}G$ into the hollow internodes of wheat stems of plants 62 days after sowing. In periods of 24–96 hours small amounts of activity were found in cellulose. Glucose derived from cellulose contained 61% of its activity in C_1. Sorbitol labelled in C_1 and succinic acid labelled in C_2 and C_3 were also tested as possible cellulose precursors. With sorbitol, activity in glucose derived from cellulose was divided between positions 1 and 6 and with succinic acid 21% was in position 1 and 26% in position 6. The conclusion was that free glucose was not a direct precursor, but became incorporated through phosphorylated derivatives largely without splitting of the carbon chain, but that some splitting of the carbon chain occurred. In support of their views the authors cite the work of Mortimer who found that after 10 seconds exposure of beet leaves to $C^{14}O_2$, cellulose became labelled but free glucose did not.

Neish (1955) has also tested $1-C^{14}$ allose, ribose and xylose and $2-C^{14}$ sedoheptulose as possible precursors of cellulose and xylan in wheat. Cut tillers of young plants (27 days after sowing) were placed in 0·5 ml of solutions of the sugars, and after the solution had been taken up transferred to water in the light. Most of the sugar taken up appeared in the methanol soluble fraction, but some incorporation into polysaccharides occurred. Glucose derived from cellulose was found to have undergone the least dilution of activity and glucose was therefore considered the most effective sugar substrate, but D-glucuronolactone was equally effective. Much of the glucose was probably incorporated without re-arrangement of the carbon skeleton. Glucose was also the best substrate for xylan synthesis and since isolated xylose from xylan was mainly labelled in C_1, it was considered to be derived from glucose by removal of C_6, and that pentoses are combined in polysaccharide only through hexose intermediates.

Xylose derived from xylan after supplying xylose labelled in C_2 and C_5 showed considerable redistribution of the isotopic carbon perhaps brought about by conversion of xylose to glucose by transaldolase and transketolase reactions, and subsequent use of the glucose in xylan synthesis. Neish has proposed the following scheme as a working hypothesis for polysaccharide synthesis in plants.

$$\begin{array}{lll} \text{G} & \longrightarrow \text{UDPG} & \longrightarrow \text{cellulose.} \\ \text{GA} & \longrightarrow \text{UDPGA} & \longrightarrow \text{polyuronides.} \\ \text{free X} & \rightleftarrows \text{UDPX} & \longrightarrow \text{xylan.} \end{array}$$

where G, GA, UDP and X represent glucose, glucuronic acid, uridine diphosphate and xylose respectively. Since UDPG is formed early in photosynthesis this scheme provides a fairly direct route for biosynthesis of cell wall carbohydrates.

Edelman, Ginsburg & Hassid (1955) used actively metabolising wheat seedlings to ensure rapid absorption of substrate, and were able to follow transformations of carbohydrates after periods of a few minutes. The seedlings, after removal from the endosperm, were suspended in 0·1 % glucose solutions, and vigorously aerated for times up to 30 minutes. The substrates were $1-C^{14}G$ and $6-C^{14}G$. Most of the activity of the ethanol extracts of the seedlings was in sucrose, and there was as well incorporation into cellulose. The distribution of activity in the moieties of sucrose and in glucose derived from cellulose when $1-C^{14}G$ was substrate are shown in Table 6.

Table 6—Percent Distribution of C^{14} in Hexoses Derived from Sucrose and Cellulose after Suspending Wheat Seedlings in a Solution of $1-C^{14}G$

(EDELMAN, GINSBURG & HASSID, 1955)

Carbon atom	Sucrose moieties glucose	Sucrose moieties fructose	Cellulose glucose
1	78·0	77·8	78·0
2 + 3	1·9	1·7	
4	0·5	2·5	3·3
5	0·0		0
6	15·6	15·3	14·5
	96·0	97·3	95·8

The same type of distribution was found with $6-C^{14}G$, namely 78 % of activity remaining in the 6 position and about 15 % appearing in the 1 position of the hexoses derived from sucrose and cellulose. The remarkable similarity in the distribution of activity in the components of sucrose and cellulose point once more to a common precursor, or metabolic pool, in which free hexose is not involved.

GINSBURG & HASSID (1955) have also reported on the formation of hemicelluloses and pentoses in wheat seedlings under the same conditions. Using $1-C^{14}G$ they found most of the activity in position 1 of pentose, little in 3 and none in 5, a finding in agreement with that already noted of NEISH.

SHAFIDEZ & WOLFRAM (1955) obtained somewhat similar results in respect of cellulose synthesis with wheat seedlings, when $1-C^{14}G$ and $6-C^{14}G$ were substrates, as shown below in Table 7. It may be noted that activity in C_2 and C_5 is higher than recorded in Table 6.

Table 7—Distribution of Radioactivity in Glucose Units of Cellulose Isolated from Wheat Seedlings after Metabolising $1-C^{14}G$ and $6-C^{14}G$

(SHAFIDEZ and WOLFRAM, 1955)

Substrate	$1-C^{14}G$	$6-C^{14}G$
	(activity in c/mol.)	
Carbon atoms All	128·5	129·1
1	60·5	28·6
2 + 5	34·3	13·5
6	38·7	92·0

From all these results it would appear that utilisation of monoses in polysaccharide synthesis involves some degree of randomisation of the carbon atoms of the chain. It is not yet possible to say whether part of the hexose was used directly and part indirectly, or whether there is re-arrangement of all the hexose used.

Lignin

It was postulated many years ago that lignin was formed by polymerisation of a C_6—C_3 or phenyl propane unit, for example coniferyl alcohol. A number of other C_6—C_3 compounds has since been tested as possible precursors, but the steps leading to synthesis of the lignin are little understood. BROWN & NEISH (1955a and 1955b) have recently reported that shikimic acid is an efficient lignin precursor in wheat and maple plants.

Uniformly labelled shikimic acid was prepared by fermentation of uniformly labelled glucose by *E. coli.* The acid was supplied in amounts of 0·10 to 0·135 m. moles through the cut ends of wheat (*Triticum vulgare*) plants and maple (*Acer negunda*) twigs. Similar experiments were carried out with phenylalanine as a C_6—C_3 compound and protocatechuic acid as an aromatic compound. After 24 hours a cell wall fraction was prepared and oxidised with nitro-benzene. The radioactivity of the vanillin and syringaldehyde formed was determined. The incorporation from shikimic acid and phenylalanine was similar, but protocatechuic acid was ineffective.

By testing a number of radioactive aromatic substances with a one carbon side chain, BROWN & NEISH have found that substances with a phenyl methane (C_6—C_1) skeleton are not in general efficient lignin precursors in wheat. In contrast substances with a phenyl propane (C_6—C_3) structure—phenylalanine, tyrosine and cinnamic acid—were readily transformed to two or more of the three lignin residues and ferulic acid was converted to the guaiacyl part of lignin. Degradation experiments indicated that the aromatic ring and at least the adjacent side chain carbon of C_6—C_3 compounds are incorporated as a unit.

The sequence of reactions proposed is the attachment of a 3 carbon side chain to a non-aromatic ring followed by aromatisation and substitution.

Translocation

Movement of sugars in plants can fairly readily be followed by the use of C^{14} tracer, and reference has already been made to the work of PAVLINOVA and TURKINA on translocation in sugar beet (see p. 145).

ARONOFF and co-workers have studied movement of assimilates in soybean plants. Thus VERNON & ARONOFF (1952) exposed the first trifoliate leaf of soybean plants 30 cm high to $C^{14}O_2$, and after 20 minutes detected activity in the stems a few cm above soil level, giving a translocation rate of about 90 cm an hour. The only compounds in the stems which had become active were sucrose fructose and glucose, and no activity appeared in the primary leaves or cotyledons. In further experiments (ARONOFF, 1955) $C^{14}O_2$ was supplied to localised areas of bean leaflets. The plants were then frozen and radioautographs obtained from the frozen material, to prevent post mortem movement. Under these conditions no activity was found in the xylem. ARONOFF summarized his results as follows.

1. Under normal conditions the flow of assimilate is greatest to the growing regions; no other movement approaches this.

2. There is no appreciable flow within a leaflet to an adjacent area or to another leaflet.
3. There is no outflow from a cut petiole in water during 24 hours, and much of the activity remains in the area which had been exposed to $C^{14}O_2$ in these circumstances. Translocation was resumed if roots were initiated.

BELIKOF (1955) examined movements of assimilate to developing bean fruits, by supplying $C^{14}O_2$ to individual leaf groups. Activity passed only into the pods attached to the treated leaf group, and during seed production activity was not found in any other part of the plant.

These experiments on translocation afford final proof of the upward movement of assimilates from leaves, of the lack of lateral movement in leaf laminae, and of the dependence of fruits on adjacent leaves for their carbon supply.

Organic Acid Metabolism

There is a number of references to the incorporation of C^{14} into organic acids, especially malic acid following exposure of leaves to $C^{14}O_2$ but detailed studies of acid metabolism using tracer C are still awaited. STUTZ & BURRIS (1951) made an extensive survey of the distribution of activity in organic acids of a number of plants after a period of assimilation of $C^{14}O_2$. They found that equilibrium in C^{14} distribution was not reached between the acids even after many hours exposure to $C^{14}O_2$. In Tomato, Tobacco, Barley and Bryophyllum 80 to 92% of the activity was found in malic acid and in Rhubarb 54%, and specific activity of the malic acid was higher than that of other acids. In the dark a high proportion of the C^{14} fixed was found in the acid fraction, but distribution between the acids varied with the plant.

KROTKOV *et al.* (1954) have found that, in light, detached tobacco leaves will take up labelled acids and that the leaf starch becomes radioactive. When formic acid or lactic acid labelled in only one C atom were given, and the glucose derived from starch degraded it was found that the 1 and 6 C atoms were more highly labelled than the 2 and 5. On the hypothesis that synthesis from CO_2 would result in even labelling, it is suggested that incorporation into starch takes place not only by reassimilation of $C^{14}O_2$ produced by oxidation of acid, but also by another pathway leading to the higher activity in positions 1 and 6.

ENZYME MECHANISMS FOR HEXOSE TRANSFER

The synthesis of oligosaccharides by transfer of hexose residues has received considerable attention since the simultaneous discovery of BACON & EDELMAN (1950) and BLANCHARD & ALBON (1950) that during the action of sucrases on sucrose oligosaccharides of greater molecular weight than sucrose were formed (see PORTER & EDELMAN, 1955). The use of labelled hexoses has contributed notably to our knowledge of the mechanism of such reactions. It was postulated that the action of sucrases was to transfer fructose residues, a conclusion which was supported by experiments with radioactive sugars (EDELMAN, 1954). Major differences in specificity of yeast and mould sucrases were also found. When these enzymes acted upon sucrose in the presence of radioactive glucose, activity was incorporated into oligosaccharides as combined glucose in each case. With mould enzyme, sucrose was the first oligosaccharide to become labelled. The reaction—a transfructosylation—may be formulated thus:

$$\mathrm{G} \longleftrightarrow \mathrm{F} + \text{enzyme} \rightleftarrows \text{enzyme} - \mathrm{F} + \mathrm{G}$$

$$\text{enzyme} - \mathrm{F} + \mathrm{G}^{*} \leftrightarrows {}^{*}\mathrm{G} \rightarrow\leftarrow \mathrm{F}$$

or

$$\mathrm{G} \rightarrow\leftarrow \mathrm{F} + - {}^{*}\mathrm{G} \qquad\qquad - {}^{*}\mathrm{G} \rightarrow\leftarrow \mathrm{F} + \mathrm{G}$$

where G→←F denotes sucrose, the arrow reducing groups and * radioactivity. This reaction may be used to synthesise sucrose labelled in the glucose moiety (see p. 137) on a preparative scale. Later in the reaction tri- and tetra-saccharides were formed which became labelled in the glucose components, supporting the theory that these compounds were formed by further fructose transfer.

$$\text{enzyme} - \mathrm{F} + \mathrm{G} \rightarrow\leftarrow \mathrm{F} \rightleftarrows \mathrm{G} \rightarrow\leftarrow \mathrm{F} \leftarrow \mathrm{F} + \text{enzyme}$$

With yeast sucrase, radioactive glucose was not incorporated into sucrose but into a new sugar, a reducing disaccharide, formed by the transfer of fructosyl residues to the 6 position of free glucose molecules. The absence of radioactivity in the reducing disaccharide, when labelled fructose was added under comparable conditions, showed that it was formed by a transfer of a fructose and not a glucose radical.

The addition of radioactive fructose to mixtures of sucrose and sucrase showed that free fructose acted as an acceptor of fructosyl groups forming radioactive fructosyl-fructose disaccharides of at least two types. As expected on the hypothesis of fructosyl transfer, none of the glucose containing oligosaccharides became labelled in the presence of labelled free fructose.

The long known occurrence of fructose polymers in plants and the evidence of presence of fructose transferring enzymes now accumulating point to the possibility that the transfer mechanism of the sucrases may merit further study.

Reference has already been made to the use of radioactive glucose in the study of the action of *B. macerans* amylase (p. 138). PAZUR & BUDOVICH (1955) have used amylotriaose in which the reducing glucose residue was uniformly labelled in an investigation of the action of crystalline α-amylase, which it has been claimed does not degrade the triaose. They observed the formation of maltose and glucose both of which were radioactive, thus demonstrating that α-amylase attacked both the glycoside linkages of the triaose. Activity of the free

Table 8—Production of Radioactive Glucose and Maltose from Amylotriaose by the Action of α-Amylase

(PAZUR and BUDOVICH, 1955)

Time (hrs.)	0	6	24
	counts per min.		
Glucose	8	185	336
Maltose	4	156	226
Amylotriaose	692	350	107
	704	691	661

glucose was always greater than that of the maltose, from which it was conclude that the rate of hydrolysis of the bond next the reducing end group was greate than that of the non-reducing end group. The activities recorded after 6 an 24 hours are shown in Table 8.

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7

RADIOISOTOPES IN THE STUDY OF MINERAL METABOLISM

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Abstract—Certain aspects of skeletal physiology and the metabolism of calcium, strontium, and iodine are reviewed. Emphasis is given to recent developments arising primarily from the use of radioisotopes in these areas of research. The subject matter is considered and integrated from two points of view: the fundamental physiology and nutrition involved and the problems arising from the potential health hazards of the fission products. It now appears that we have a reasonable understanding of what happens under varying conditions to these radioisotopes after they enter the body. Also, it is possible to estimate the retention by man and the entrance into edible products of the animal when the dietary intake is known.

1. INTRODUCTION

THIS review has been limited to aspects of skeletal physiology, and the metabolism of calcium, strontium, and iodine; these are fields of investigation to which major contributions have been made by the use of radioisotopes within recent years. The research activity and interest in these subjects are reflected by the numerous papers at the Geneva Conference that were concerned with these matters. The interest ranges from the fundamental to the intensely practical, from basic studies of the physiological and metabolic behaviour of these elements to the problems concerned with the potential health hazards from assimilated fission products.

In presentation, there first was an attempt to describe general concepts where these have been recently developed. Controversial issues and divergence of interpretation have been brought out with the hope of inciting further work.

An excellent reference source that provides coverage of the entire biological field is entitled, "Isotopes—An Eight Year Summary of United States Distribution and Utilization," published by the United States Atomic Energy Commission. This report lists the titles of over 7 000 individual papers dealing with isotopes and their applications. Details of techniques are available in the usual texts: CALVIN *et al.* (1949), GLASCOCK (1954b), COMAR (1955), HEVESY (1948), KAMEN (1951), SIRI (1949), WHITEHOUSE & PUTMAN (1953).

Grateful acknowledgment is made to our many colleagues for stimulating discussions which must inevitably leave their mark in our thinking and writing. Thanks are due also to those who have so kindly allowed the use of experimental results not yet appearing in print.

2. SKELETAL PHYSIOLOGY(1)

In the years before World War II, the growth of knowledge of skeletal dynamics and bone formation was tediously slow. The advent of the atomic

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(1) The section on Skeletal Physiology is contributed by and is essentially the paper of W. F. NEUMAN entitled, "The Use of Isotopes in Study of Skeletal Physiology and Metabolism," presented at the International Conference on the Peaceful Uses of Atomic Energy.

age simultaneously provided the impetus and helpful study-aids for renewed and vigorous research in this important branch of physiology. The impetus was the potential exposure of large segments of our population to the radioactive bone-seeking transuranic elements and fission products; the study-aids were the inexpensive pile-produced radioisotopes of all of the important bone elements.

The earliest investigators approached the problem directly by administering radioisotopes orally or parenterally to intact animals and determining thereafter the gross patterns of distribution and excretion. The interpretation of these data proved very difficult because of the complexity of the physiological events involved. It was soon recognized, for example, that a dose of radiocalcium was almost completely deposited in the skeleton within an hour—too much and too fast to be explained as the result of growth or accretion of new bone mineral. It was postulated that an exchange of the radiocalcium for normal calcium in pre-existing bone must have occurred, but careful studies of isolated, model systems *in vitro* were necessary to establish the mechanisms involved. Such experiments (bone mineral exposed to synthetic buffers containing radioisotopes) did more than establish the exchange substitution of radiocalcium for normal calcium. Attention was focused on the importance of the surface chemistry of the tiny bone crystals and our understanding of the nature of the bone mineral was thereby greatly clarified. The presence in bone of such physiological ions as sodium, magnesium, carbonate, and citrate, and such nonphysiological ions as uranyl, lead, radium, and fluoride is now established as principally the result of a passive, physico-chemical exchange displacement of one or more of the normal lattice ions: calcium, phosphate, or hydroxyl.

As knowledge of the mechanisms of isotope-fixation grew, the histologists perfected autoradiographic techniques. With this tool, the histological distribution of radioisotopes deposited both *in vivo* and *in vitro* was determined. A beautiful correlation between newly-forming bone elements and intensive deposition of isotopes was invariably observed. This stimulated an intensive application of physical techniques, such as electron microscopy, microradiography, and low angle X-ray diffraction. As a result, the size and habit of the bone crystals are now known and a close interrelation between the collagen fibres and the crystals has been established. Autoradiography has also improved our understanding of the morphological patterns of the growth of bone. Radiocarbon, radiosulphate, and, more recently, plutonium have proved to be wonderfully permanent "markers" of uncalcified organic matrix, showing little if any redistribution subsequent to the initial deposition at the time of injection.

Increased knowledge of bone ultrastructure, growth patterns, and the chemistry of bone mineral has notably changed our concepts of the calcification process. Indirectly, then, radioisotopes have led to a discarding of "precipitation" schemes and have initiated a search for the mechanism by which the collagen fibres of ossifying cartilage and osteoid uniquely "seed" crystal formation from the body fluids; it is remembered that the concentration of salts in the body fluids is below that required for spontaneous precipitation.

Although only a beginning has been made, enough is now known of the mechanisms of normal skeletal metabolism and growth to expect in the near future fruitful applications of isotopic techniques in pathological conditions, such as osteoporosis, osteomalacia, rickets, and fracture. In the discussion that follows a summary is presented of current concepts of normal skeletal physiology as derived principally from radioisotope data, enlarged and supplemented by adjunctive techniques and disciplines. Some general references covering this subject are: McLEAN & URIST (1955), SPENCER & UHLER (1955), WEINMANN & SICHER (1947) and ARMSTRONG (1952). References of particular value are the symposiums entitled, "Recent Advances in the Study of the Structure, Composition and Growth of Mineralized Tissues", published in the *Annals of the New York Academy of Science* (1955) and the *Transactions of Conferences* 1–5 on "Metabolic Interrelations", published by The Josiah Macy, Jr. Foundation, New York (1949–1953).

A. *Composition and Structure of Bone*

Bone is comprised of four kinds of material: inorganic crystals, water, gel-like polysaccharides, and protein fibres.

As seen in the electron microscope, the mineral crystals of bone are small flat tablets of varying dimensions (100–1 000 Å long and wide and 10–100 Å thick). This minute size accounts for the fantastically high specific surface of bone mineral—over 100 m^2/g. The crystals are so thin that from $\frac{1}{10}$ to $\frac{1}{4}$ of all the ions present reside in the crystal surfaces or in the layers immediately beneath. Bone crystals from all sources give an X-ray diffraction pattern that is characteristic of a structure the mineralogists have termed the hydroxy apatite crystal lattice. This lattice, or spatial arrangements of ions, is common to a whole series of solid phosphates whose molar ratio of Ca:P varies from 1·3 to 2·0. The lack of strict stoichiometry has been ascribed to: (a) the isomorphic substitution of water or hydronium ions, or both, for calcium, (b) the common occurrence of numerous faults, defects or voids within the lattice, and (c) surface-exchange and surface-adsorption. The prototype or model lattice of hydroxy apatite exhibits a Ca/P molar ratio of 1·66 and the smallest repeating unit of structure or unit cell may be represented: $Ca_{10}(PO_4)_6(OH)_2$. In bone, the crystals contain variable amounts of other ions found in the circulating fluids, principally: Na^+, $CO_3^=$, citrate$^{\equiv}$, Mg^{++}, F^-. These ions enter the crystals by an exchange displacement of the normal lattice ions residing at the crystal surfaces.

Recently it was shown that hydroxy apatite crystals become highly hydrated when suspended in an aqueous medium. Crystals having a specific surface of 68 m^2/g bind 0·8 g H_2O/g or a volume $2\frac{1}{2}$ times that of the crystals themselves. The nature of this hydration shell is not clearly understood. Electrostatic effects are at least partly responsible since the crystals also bind polar organic molecules such as the alcohols. Much of the water present in bone is water of hydration, not interstitial fluid as had been previously supposed. In old, established, cortical bone nearly all of the water is in this form, not separable by high-speed centrifugation and unavailable for exchange with deuterium oxide.

The polysaccharide gel is a poorly understood constituent of bone. Since this gel is the continuous phase, it has been termed the ground substance. These polysaccharides are polymers of glucuronic acid and hexosamines, and constitute a little less than 5% of the dry, fat-free weight of bone. Both sulphated and unsulphated polymers are present. It seems reasonable to assume that the degree of polymerization directly affects the permeability and the flow of fluids.

Nearly all of the protein present is in the form of the highly organized fibres of collagen. These fibres are variable in size, averaging about 800 Å in width, with a characteristic dense-staining cross banding along the length of the fibres at approximately 640 Å intervals. Low-angle X-ray diffraction and electron microscopy have shown that the apatite crystals lie in or on the collagen fibres. The c-axes of crystalline symmetry are aligned parallel to the longitudinal axes of the associated fibres. In addition to the general references, the papers of Robinson & Watson (1952) and Martin (1953) are of interest.

B. *Growth and Development of Bone*

A most important feature of bone growth is the maintenance of structural strength during the growth process itself. This is accomplished by continuous and simultaneous erosion and deposition. Thus, the shafts of the long bones enlarge in diameter through deposition of bone on the outer surfaces. Erosion occurs simultaneously on the inner surfaces, thereby enlarging the marrow cavity. Similarly, these bones lengthen by deposition at the ends while support is provided by the recently calcified bridges just inside the ends. These bridges are soon eroded by capillary invasion as the marrow cavity moves outward. This unique feature of erosion and deposition is not limited to the growth period but continues in isolated areas, e.g. the Haversian systems within the fully grown bone.

Although experimental data are meagre, there is presumptive evidence for the following generalizations about the ultrastructure of the newly forming areas: the crystals are small, imperfect, and highly hydrated, and the polysaccharide gel is incompletely polymerized; the gross architecture is frequently spongy in character, providing ready access to the circulation. All of these factors combine to render the newly formed areas more reactive than the dense, poorly hydrated areas of well-established, mature bone. Because of this correlation between chemical reactivity and growth activity, bone-seeking elements all tend to concentrate in the many regions of growth in the infant and in the discrete, reforming areas, i.e. the Haversian systems, in the adult. Lacroix (1951), Owen *et al.* (1955), and Ham (1952) may be referred to in connection with growth and development of bone.

C. *Deposition of Radioisotopes*

A group of elements have been shown to concentrate *in vivo* specifically in the organic matrix, or osteoid, of forming bone.(2) The specificity is in itself remarkable. Even more amazing is the lack of "dissociation" of the deposited material. Shortly after deposition, one member of the group may in part displace another, but usually the deposited material remains for the entire life of the bone with scarcely any decrease in concentration. As a group, these elements (americium, plutonium, yttrium, barium, zirconium, cerium, gallium, niobium, lanthanum, praseodymium, neodymium, promethium, samarium, europium, neptunium, actinium) show a pronounced tendency to form colloidal hydroxide gels at physiological pH. There is, at present, no information available on the underlying mechanisms responsible for the deposition. Clarifying research is urgently needed. There is autoradiographic evidence that significant amounts of uranyl ion and strontium also may be held by the organic matrix. In this regard, it may be noted that the Ca^{45}-binding capacity of demineralized bone sections *in vitro* can be correlated with the presence of sulphated polysaccharides.

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(2) Jowsey, Sissons, & Vaughan (1955) have recently reported histological evidence that yttrium tends to be associated with bone surfaces in regions of resorption and inactivity; the yttrium was not observed in the osteoid matrix in areas of growing bone.

Another group of elements deposits mainly in the mineral phase of bone in a manner more clearly understood. This group (calcium, strontium, radium, magnesium, uranium, lead, and perhaps beryllium and thorium) enters the surfaces of the mineral crystals by an exchange-displacement of normal calcium ions. This exchange involves a three-step process: diffusion into the outer hydration shell, surface exchange, and recrystallization. Recrystallization is considered as a slow penetration of ions into the crystal interior by a thermal process mediated by faults in the crystal structure. Although these three main surface reactions of bone mineral have been primarily demonstrated and clarified by studies of model systems *in vitro*, they are known to be operative in the living animal. *In vivo*, these processes are modified and influenced by physiological conditions. Therefore, the biological fate of an injected cation, for example, calcium 45, cannot be accurately described in simple physical terms of diffusion, exchange, and recrystallization.

Immediately following intravenous administration, radiocalcium leaves the blood very rapidly, and the decrease in circulating radioactivity with time follows a course that can be described as a series of exponential decay curves. The first, most rapid loss can reasonably be assumed to be diffusion into the extracellular spaces. The less rapid losses can perhaps be correlated with the diffusion into the skeleton and the exchange of radioisotopes for normal calcium in the bone crystal surfaces. Because of the enormous bulk of the skeletal calcium in comparison to that circulating, most of the isotope is found in the skeleton within an hour after administration. These events are all consistent with our understanding of diffusion and exchange. In the succeeding hours, it would be expected that the radiocalcium would be further incorporated into the bone by recrystallization and new crystal formation.

Since it was first described, the process of recrystallization has been a source of confusion and controversy. While the process can be easily demonstrated *in vitro* and *in vivo*, its quantitative physiological significance has been difficult to ascertain. No final assessment can yet be made, but inferential evidence indicates the following: in areas of growth, the newly formed crystals are hardly crystals at all. Rather, they behave more like gel-like masses in which nearly all the ions are capable of rapid displacement. In a short time, however, they perfect themselves by recrystallization and assume the stable configuration of true bone crystals. Recrystallization decreases rapidly in rate and quantity with the age of the animal. In the adult animal, it is unlikely that more than 1 or 2% of the total skeletal calcium can exchange by recrystallization, perhaps even less. In the weanling rat, however, over 10% of the crystals present must be less than one day old. Under such circumstances, recrystallization must be of quantitative importance.

Isotopes of calcium and phosphorus (as phosphate), and other ions capable of entering the apatite lattice, are deposited during the accretion of new crystals. Whatever the mechanisms involved, such a deposition is not ion exchange in the ordinary sense of the word. In isotope injection experiments of short duration, the passive physico-chemical exchange reactions are dominating, modified only by the physiological state of the skeleton. In *young* animals, however, the growth processes rapidly assume dominance. The infant rat serves as an excellent example. It adds as much as 15% to its skeletal weight in a single day. Because of the complicated way in which bone grows, it seems reasonable to assume that a net increase of 15% is accompanied by an equal amount of accretion, resorption, and translocation. Thus, 30% of the skeletal mineral is directly concerned in events associated with growth in the preceding 24 hours. After a week nearly all the skeleton must have been so involved.

All of these processes—diffusion, exchange, recrystallization, and new crystal formation—decrease in rate and quantity with age. In fact, only a fraction of the adult skeleton seems able to participate in even simple exchange. Attempts have been made to determine this fraction of "available" skeleton in various animals. Radiocalcium is of little value for such a measurement because it is difficult to ascertain how much enters the body by new accretion and by recrystallization.(3) A cation, such as sodium, that is incapable of entering the lattice interior, is more suitable. By measuring the percentage of skeletal sodium that is readily exchanged with injected radiosodium, one obtains directly an estimate of the "available" fraction of total skeletal mineral. A number of such estimates are published and vary from about one-quarter in adult animals to about three-quarters in the infant rat.

The known physiological variables that affect exchange phenomena in the skeleton are: (a) accretion, resorption, and redeposition in growth; (b) resorption and redeposition in Haversian systems in adult remodelling; and (c) the gradual aggregation and polymerization of organic and inorganic elements in established bone. Some general references on deposition of radioisotopes in bone are as follows: AMPRINO (1952), BAUER (1954), COMAR, LOTZ & BOYD (1952), HAMILTON (1947), HEVESY (1948), HOECKER & ROOFE (1951), KIDMAN *et al.* (1952), NEUMAN & NEUMAN (1953), MACDONALD *et al.* (1953), and THOMAS *et al.* (1952).

D. *Mobilization of Radioisotopes*

While the occurrence of skeletal exchange reactions can be easily demonstrated, the question of physiological interest is whether *net* transfer of ions from body fluids to bone, and the reverse, can occur without a complete dissolution of crystals by cellular processes. For example, about half of the

(3) TOMLIN, HENRY, & KON (1955) have described an interesting technique for estimation of the exchangeable fraction of cortical bone calcium in young rats, that gave a lower value (0·2%) than those found in the literature. The method is based on the comparison of specific activities of bone samples from rats that had been fed a labelled ration during the period of bone formation and those that had been fed this same ration for a short time only.

body sodium resides in the skeleton of the adult rat and 30% of this can readily exchange with sodium in the circulation. Will this exchangeable sodium readily leave the bones to support the blood level during sodium depletion?

Recent data show that greater amounts of sodium are drawn from the skeleton than from the extracellular fluids following sodium depletion in the rat by a 4-hour intraperitoneal dialysis with NH_4Cl. Similarly, an extremely rapid transfer from bone of an amount of calcium equal to three times that in the extracellular fluids was demonstrated by transfusion experiments in dogs. The reverse transfer, from blood to bone, cannot be shown so elegantly, but may be inferred from new data where some 40 mg of injected calcium was taken up by the skeleton of a dog in less than an hour. Similar results were obtained when strontium salts were injected.

Thus, it is apparent that a large net transfer of ions can occur from blood to bone and bone to blood. Explanation of these observations by an ion exchange mechanism only offers many uncertainties. For example, in the sodium depletion experiments, it is not possible to explain the loss of bone sodium as a direct reversal of the postulated calcium displacement. The animals would have died in tetany as calcium was drawn from the blood to replace the needed bone sodium. Nor can it be assumed that sodium ion left the bone by itself; this would result in electrostatic imbalance. In this same experiment, these animals had become acidotic. Was sodium accompanied by a loss of bone carbonate? In all probability this occurred. The transfer of both sodium and carbonate ions from solid to solution cannot be considered conventional *ion exchange* but rather more like a simple dissolution or desorption process.

The mobilization of skeletally deposited radioisotopes is an important problem of the atomic age. Thus far, most attempts to reduce such a skeletal "burden" by means of chelating agents and other chemical compounds, drugs, and hormones have been relatively unsuccessful. The reason for this apparent failure seems to reside in the irreversibility of the processes of bone formation. In the adult, "bone-seekers" are deposited principally in the newly forming Haversian systems at the time of entry into the body. With the passage of time, these new Haversian systems rapidly mature and become inactive, inert, and impermeable. The radioisotope thus becomes entrapped, isolated from contact with the circulating fluids. None of the agents yet tried possesses the ability to demineralize only those Haversian systems that were forming at the time of the radioactive-exposure. Thus, at the moment, there seems to be no alternative other than complete demineralization of the entire skeleton, a therapy fraught with danger and difficulty. This matter of removal of bone-seekers is discussed from a practical standpoint in the next section.

E. *Treatment for Removal of Radioisotopes from Bone*

The basis for the following discussion is the review by SCHUBERT (1955) on practical measures for removal of bone-seeking radioisotopes from the body.

(1) *Minimization of absorption*—Absorption of a soluble radioisotope, such as radiostrontium, from the gastro-intestinal tract is so rapid that treatment must be started immediately if it is to be effective. After oral ingestion,

there are three obvious approaches to treatment: (a) use of an emetic, (b) minimization of absorption by use of precipitating agents, and (c) use of cathartics.

With elements that form insoluble hydroxides at physiological pH, treatment may not be needed, except for a cathartic, because absorption from the gastro-intestinal tract is very small. For example, the absorption of plutonium and the rare earths is usually less than 0·01%. Therefore, for plutonium, polonium, cerium, and praseodymium, the use of precipitating agents can probably be omitted. When very large amounts are swallowed, it might be advisable to decrease the solubility of the radioisotope by raising the pH of the gastric juice by oral administration of nonsystemic antacids, such as magnesium oxide, "Sippy powders", or aluminium hydroxide. An antacid in combination with a cathartic would appear to be the best approach. With the rare earth elements, which are more soluble than plutonium, it is possible that oxalate-containing foods, such as rhubarb and spinach, or a finely divided carboxylic type cation exchange resin, such as Amberlite IRC-50, may bind the radioisotopes. Following accidental oral ingestion, it may be advisable to avoid the use of solubilizing chelating agents and other substances that promote absorption. Non-specific adsorbents such as zirconium oxide might be of value.

The alkaline earth group of elements are absorbed to a significant extent from the gastro-intestinal tract. Since radium and strontium form insoluble sulphates, the treatment of choice would appear to be magnesium sulphate in combination with a laxative and possibly an antacid as well.

The inhalation of soluble forms of radium and strontium is nearly equivalent to an intravenous injection. Absorption from the lungs is so rapid that treatment is largely similar to that required following injection into the bloodstream. However, the upper nasal passages do retain some of the radioisotope. In one situation in which a human being inhaled $Sr^{90}Cl_2$ from an aqueous solution, the upper nasal passages were sprayed with 0·25% neosynephrine solution with the thought that vasoconstriction might lessen absorption and facilitate washing. The use of nasal swabs prior to irrigation should be helpful.

Following inhalation of insoluble types of radioactive substances, retention in the lung depends on the particle size of the inhaled substance. The first step is to swab thoroughly around the vestibule of the external nares. In addition, consideration should be given to the fact that the insoluble material in the lungs may reach the mouth and be subsequently swallowed.

Following puncture of the skin with an object contaminated with radioisotopes, excision and irrigation of the wound site appear most practical.

(2) *Interference with calcium metabolism*—As already noted from theoretical considerations, one would not expect decalcification therapy to be generally effective. For example, a low calcium diet plus ammonium chloride, thyroid extract, and parathyroid extract may increase the excretion of radium, strontium, and calcium but at too slow a rate to have any appreciable effect on the residue left in the body. Of course, prompt treatment of acute exposures may be of some benefit. HOECKER (1955), studying radium deposition in lactating rats, showed that a negative calcium balance induced before or immediately after exposure can markedly reduce retention of such a

radioisotope. Decalcification therapies have no observable effect on the excretion rates of plutonium and rare earth elements.

(3) *Chelates and other complexing agents*—When a metal is complexed by different chemical compounds, usually organic in nature, a poorly dissociated molecule is formed; when the complex metal is part of a ring system, the term "chelate" is applied. A chelated metal ion does not undergo its normal chemical reactions except with substances having a stronger affinity for the metal ion than does the chelating agent. A water-soluble and easily diffusible chelate is readily excreted. Thus, in principle, the chelating or complexing agent removes the metal ion from combination with cellular constituents with subsequent elimination of the complexed metal from the body.

Desirable properties of complexing agents are as follows: (a) forms complexes that are water-soluble and readily diffusible, (b) does not react with normal body constituents (calcium is usually the problem), (c) has low toxicity, (d) is well absorbed from the gastro-intestinal tract, and (e) is not metabolized. The three principal agents at present are EDTA (ethylenediaminetetra-acetic acid), BAL (2, 3-dimercaptopropanol, British anti-lewisite) and sodium citrate.

EDTA has been found to pass through the body unchanged after parenteral administration and to mix rapidly with nearly all the body water. As the sodium salt, relatively small doses of 20 to 50 mg/kg produce death in dogs because of hypocalcaemia; as the calcium salt, dogs can tolerate up to 4 000 mg/kg. In human beings up to 2·5 g of Ca EDTA have been given in 250 ml of saline by slow intravenous drip twice daily; ingestion at levels of 30 mg/kg has caused some nausea and abdominal cramps. Repeated high-level dosage of either the sodium or calcium salt has been reported to have caused kidney damage.

Studies with man and animals have shown that EDTA is significantly effective in removing from tissues previously deposited radioisotopes of plutonium, lanthanum, yttrium and, in general, the rare earths and trivalent transuranic elements. EDTA is most effective when administered soon after exposure (within a few days). When prolonged treatment is started a month after exposure, substantial but not maximal reductions in body burden can be attained. When treatment is delayed for a year or more, significant effects are not to be expected. EDTA has not been effective against the alkaline earths: radium, strontium, and barium.

BAL has been found to increase the faecal excretion of previously injected polonium from rats. However, BAL has no effect on substances that do not react with sulphhydryl groupings, as shown by studies with radium, strontium, phosphorus, plutonium, and yttrium.

The effect of citrates on excretion of plutonium and rare earths is relatively insignificant even under optimum conditions. With thorium and strontium, early administration of sodium citrate gave surprisingly large effects. It was shown, however, that the increased urinary excretion of strontium that followed the citrate treatment was derived mainly from the circulating strontium; apparently, the administration of sodium citrate did not remove deposited strontium from the bone. These results suggest that, if the metabolic destruction of injected citrates could be reduced or if an elevated citrate level could otherwise be maintained in the tissues and blood, it would be possible to improve the ability of citrates to accelerate the excretion of radioisotopes. Under these conditions, the citrates could be of value in the treatment of many radioactive poisons. In principle this is possible by administering metabolic inhibitors that block the normal destruction of citrates *in vivo*. These drugs include non-lethal doses of sodium fluoroacetate, which cause large and sustained elevations of citrate levels in various soft tissues. Guanidine and guanidine derivatives increase the blood citrate levels two- to threefold. Finally, vitamin D appears to accelerate citric acid production in bone.

(4) *Colloidal carriers and zirconium salts*—Of various salts studied for this purpose, zirconium citrate was found to cause marked increases in excretion of many different radioisotopes. The mechanism was explained as follows: after the entrance of zirconium salts into the blood-stream, colloidal aggregates are formed by the precipitation of zirconium hydroxide above pH 2; the circulating radioisotope is adsorbed by these aggregates and its disposition is governed by the fate of the circulating colloidal particles. Zirconium citrate treatment has been used primarily for plutonium and yttrium; the method has been found more effective in dogs than in rats.

The effect of zirconium is greater when administered shortly after the injection of the radioisotope, just as when chelating agents are used. An important difference, however, is that a single injection of zirconium given before or after administration of the radioisotope will continue to influence the excretion or retention of the radioisotope for periods of days or weeks, depending on the initial dose of the salt. Preliminary studies with rats have indicated that a combination of zirconium citrate and Ca EDTA may offer some advantage.

(5) *Miscellaneous approaches*—Attempts to treat radioactive poisoning by several other methods have been made. None of these has had a significant effect, at least for the specific radioisotope tested. They include:

1. Induced diuresis by injection of hypertonic saline into dogs (tested with plutonium).
2. Disturbance of the organic matrix of bone by induction of scurvy using a scorbutogenic diet (tested with plutonium, strontium, and yttrium).
3. Use of a protein-free diet to disturb bone protein (tested with plutonium and strontium).
4. Causation of bone resorption with subsequent use of a diet calculated to restore the bones with new, non-radioactive elements with the hope that the new bone would act as a mass absorber to prevent radiations from deposited plutonium from reaching living cells.
5. Use of endocrine changes exemplified by cretinism produced in rats by thyroidectomy at birth. The resulting hypothyroidism inhibited the deposition of radiostrontium in the skeleton. Growth hormone and thyroxine were administered either alone or together to the hypothyroid rats. These treatments increased the skeletal uptake of strontium to levels that sometimes exceeded those of the normal controls.

It is conceivable that stimulation of the phagocytic cells of the reticulo-endothelial system may enable them to cope more adequately with radioactive particles. Substances, such as oestradiol, benzoate, and choline, stimulate these cells; also choline enhances the rate of disappearance of colloidal $CrP^{32}O_4$ from the blood. Some general references are: FOREMAN (1953), FOREMAN, FUQUA & NORWOOD (1954), HART & LASZLO (1953), SCHUBERT & WHITE (1950), and SCHUBERT (1951).

3. CALCIUM METABOLISM

A. *Interpretation of Bone Studies with Radiocalcium*

At present there appears to be a divergence of opinion regarding the processes by means of which radiocalcium (Ca*) ions leave the blood to become incorporated in the skeleton. The importance of this issue is concerned with the use of Ca* to measure new bone salt formation. If Ca* ions leave the blood and enter the skeleton only by bone salt deposition, then the amount of this deposition can be measured from the following equation:

$$M = \frac{\mathrm{Ca}^*_b}{S_M} \qquad (1)$$

where M = mass of calcium incorporated into new bone salt of sample during the time between radioisotope dosage and removal of bone sample

S_M = average specific activity of serum calcium during this time interval

Ca^*_b = amount of radiocalcium in the bone sample.

The attraction of this type of calculation lies in the difficulty of estimating new bone salt deposition by any other method; direct measurements from net increases in bone weight are confounded by the bone resorption that occurs simultaneously, described as follows:

$$\text{New bone} = \text{net increase} + \text{bone resorbed}. \qquad (2)$$

However, if significant amounts of Ca* ions enter the bone by processes such as exchange, equation (1) is not valid.

Since the first observations of HEVESY (1948) in which he used labelled phosphate, the concept of exchange gained dominance. Statements by various workers appearing in the literature might be considered too sweeping in denying the usefulness of radiocalcium and radiophosphate for studying skeletal metabolism. Within recent years attention has been directed toward the importance of new bone salt deposition in accounting for the entrance of Ca* ions into bone. As an example of this approach, reference is made to the work of CARLSSON and co-workers.

In brief, BAUER, CARLSSON & LINDQUIST (1955) have reported the following equations:

$$\mathrm{Ca}^*_{\mathrm{obs}} = \mathrm{Ca}^*_E + \mathrm{Ca}^*_A - \mathrm{Ca}^*_R \qquad (3)$$

where $\mathrm{Ca}^*_{\mathrm{obs}}$ = total amount of Ca* present in a calcified tissue

Ca^*_E = amount of Ca* present in the exchangeable fraction of the bone salt

Ca^*_A = amount of Ca* incorporated into the non-exchangeable fraction of bone salt through accretion

Ca^*_R = amount of Ca* removed from calcified tissue through resorption

$$\mathrm{Ca}^*_E = E \times S \qquad (4)$$

where E = amount of Ca in the exchangeable fraction of bone salt
S = specific activity of serum Ca

$$\text{Ca*}_A = A \times T \times S_M \tag{5}$$

where A = rate of Ca accretion
T = time between Ca* administration and observation
S_M = average specific activity of serum Ca during this time interval (Note similarity of equation 1 and equation 5)

Equation (3) can then be expressed as:

$$\text{Ca*}_{\text{obs}} = E \times S + A \times T \times S_M - \text{Ca*}_R \tag{6}$$

Equation (6), according to BAUER *et al.*, can be simplified by choosing a time-interval such that S approaches 0 and Ca*_R is small. Under such conditions

$$\text{Ca*}_{\text{obs}} = \text{Ca*}_A = A \times T \times S_M \tag{7}$$

If measurements are made at two time-intervals (designated by subscripts 1 and 2) then the following equation is obtained:

$$\frac{\text{Ca*}_{1-\text{obs}} - A \times T_1 \times S_{1-M}}{\text{Ca*}_{2-\text{obs}} - A \times T_2 \times S_{2-M}} = \frac{S_1}{S_2} \tag{8}$$

These equations were used with data from rat studies to calculate the quantities as shown in Table 1. It is interesting to note that in the whole tibia the rate of resorption was about three times the rate of net increase.

A possible uncertainty in this approach may be considered in terms of equation (3). At short time intervals this equation is valid. However, at longer time intervals much of the Ca* originally in the exchangeable fraction finds itself in the non-exchangeable fraction; according to equation (3) all such Ca* ions are lumped into the new bone salt fraction for the calculations by equations (5) and (7). The issue then is, do the Ca* ions that are initially deposited by exchange become subsequently incorporated into the non-exchangeable fraction by a process that is governed by equations (1) and (5)?

Table 1—*Calcium Metabolism in the Two Tibias of a Young Rat*

	Whole tibias	Ends	Shafts
Ca content, mg	66	36	30
Net increase, mg Ca per hour	0·04	0·02	0·02
New bone deposition, mg Ca per hour	0·17	0·14	0·03
Resorption, mg Ca per hour	0·13	0·12	0·01
Exchangeable fraction, mg Ca	2·0	1·7	0·3

(From BAUER, CARLSSON & LINDQUIST, 1955.)

The values in Table 1 do not appear unreasonable, and it is not yet possible to ascertain the extent of error, if any, which is thus introduced. The physiological limitations, however, of such equations should be kept in mind.

Regardless of the interpretation, there seems to be general agreement as to these experimental observations:

(a) When Ca* ions enter the blood some are found in bone within minutes as shown by simple analysis. A fair proportion of these ions are in the exchangeable fraction as shown by the specific activity of serum and the amount of calcium in the exchangeable fraction (see equation 4).

(b) By the same token, after a matter of hours, a large proportion of the Ca* ions are in the non-exchangeable fraction; that is, they are irreversibly fixed.

(c) After several hours, when steady state has been attained in movement of Ca* between serum and bone, the addition of Ca* to the skeleton can be then accounted for by new bone salt deposition.

Some of the studies needed for better understanding of these issues are mentioned:

(a) An accurate measurement of the rate at which Ca* ions enter the skeleton from the body fluids; this may have to take into account rates of blood-flow to bone as a limiting factor.

(b) Clarification of the processes by means of which exchangeable Ca* ions in the skeleton become irreversibly fixed.

(c) Independent estimation of rates of bone resorption.

B. *Faecal Endogenous Loss of Calcium and Phosphorus*

Attention is now directed toward the broader aspects of calcium metabolism, primarily concerning absorption and excretion. Fig. 1 presents a simple model of calcium ion movement in the body. It is considered that the circulating calcium in the blood comes into contact with the surfaces of a certain portion of the bone mineral and exchanges with the surface calcium; also blood calcium is removed to form new bone and, simultaneously, bone is resorbed to supply calcium to the blood. New calcium enters the blood from the gastro-intestinal tract and blood calcium is lost by urinary and faecal excretion.

It is clear that after entry of Ca* into this system and attainment of steady state, then the removal of Ca* from the body will be governed by its dilution with stable calcium from the diet and the excretory losses. Urinary losses can be measured directly but estimations of net absorption and faecal loss from the body require determination of the faecal endogenous loss.

The determination of faecal endogenous loss is also important from the standpoint of nutritional studies. Such information allows direct estimation of: (a) the proportion of the element in a given feed that is unavailable to the animal, and conversely, the net digestibility; (b) the maintenance requirements; and (c) the actual body losses as they may be affected by such variables as feed levels and age.

Modifications of the isotope dilution method for measurement of faecal endogenous losses have been described by HEVESY (1948) for phosphorus in man, KLEIBER and co-workers (1951, 1955) for phosphorus in livestock, COMAR *et al.* (1953) and VISEK *et al.* (1953a) for calcium in cattle, and BLAU *et al.* (1954) for calcium in man. In principle the plasma calcium, which is the source of endogenous calcium, is labelled. The labelled calcium that then reaches the faeces by the endogenous route is diluted with the unabsorbed calcium of the diet. The extent of this dilution is calculated from the ratio of faecal to plasma specific activities, and the proportion of faecal calcium contributed by each source is thereby determined.

In earlier work multiple injections of radioisotopes over periods of day were used to label the plasma. However, KLEIBER (1955) with P^{32} anc LENGEMANN, MONROE & COMAR (1955) with P^{32} and Ca^{45} have simplified the procedure by the use of a single injection. From experience over several years

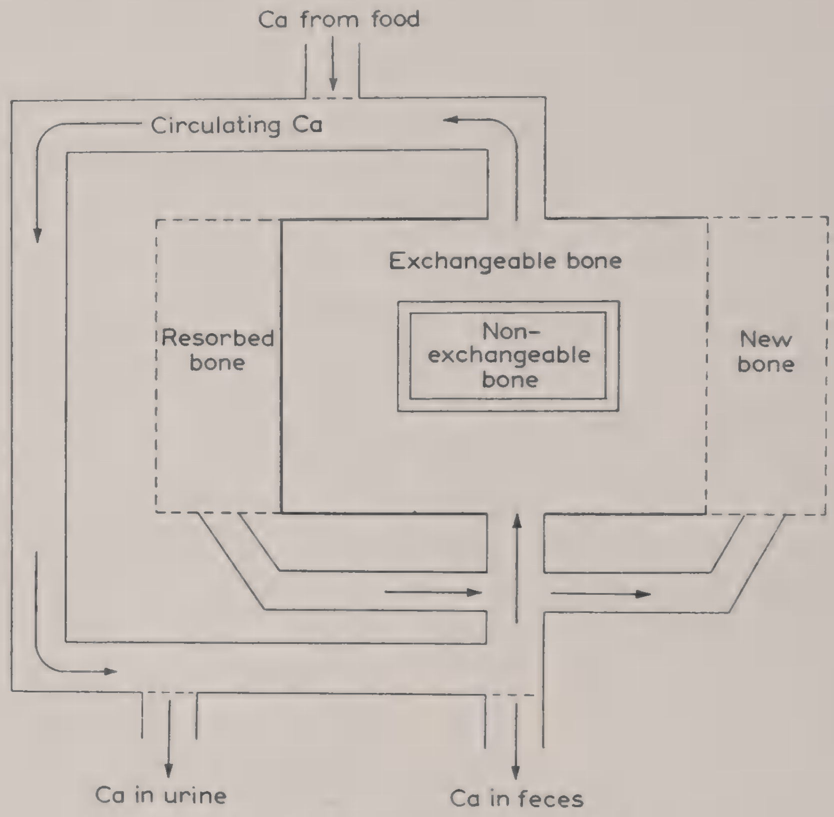

Fig. 1. Simple model of calcium ion movement in body.

the following procedures and schedule for simultaneous determination of endogenous faecal calcium and phosphorus in cattle have been found to offer a compromise of ease and reliability:

(a) The animal is conditioned to the metabolism stall and to eat normally when placed on experiment. This stall is arranged for separate and quantitative collection of faeces.

(b) When the animal is reacting normally, as judged by food consumption and nature and amount of excreta, the solution containing P^{32} and Ca^{45} is injected either subcutaneously or intramuscularly. Usually 1 millicurie of Ca^{45} and 0·3 millicurie of P^{32} per 100 pounds body-weight are given.

(c) On the sixth day after dosage a blood sample of about 50 ml is taken.

(d) On the seventh day after dosage the faecal collections are started. Records of total excretion are kept over the next five days, and from each day's collection a representative faecal sample is taken for analysis. Alternatively, a composite faecal sample representative of the five days' excretion can be used for analysis.

(e) On the eleventh day after dosage another blood sample is taken.

(f) Samples are analysed for total calcium, total phosphorus, and for Ca^{45} and P^{32} by the differential absorber method.

Calculations are made as follows (the equations also apply to phosphorus):

$$\frac{\text{Average specific activity of faeces}}{\text{Average specific activity of plasma}} \times \text{daily faecal Ca} = \text{endogenous faecal calcium} \tag{9}$$

$$\frac{\text{Ca intake-faecal Ca} + \text{endogenous faecal Ca}}{\text{Ca intake}} \times 100 = \text{net digestibility} \tag{10}$$

An important point is that the mean of the 6th and 11th day specific activities of the plasma represents the average value over this time period. Thus, only one injection and two withdrawals of blood are required, which minimizes annoyance to the animal.

An uncertainty of interpretation of faecal endogenous calcium values estimated by the isotope dilution method is related to the extent of exchangeability between calcium in the circulation and in the gastro-intestinal tract. If there were complete exchangeability, then all faecal calcium would be classified as endogenous by this method. The data to be presented indicate that such is not true. However, THOMAS, LITOVITZ & GESCHICKTER (1954) have presented evidence with rabbits that some exchange does occur. It must be remembered then that the faecal endogenous values may represent both actual secretion of the element into the gastro-intestinal tract and interchange between blood and tract. Further work is needed to estimate the relative magnitude of each process.

Attention is called to a calculation from these data of total digestive juice calcium, TDJ Ca (BLAU *et al.*, 1954). The TDJ Ca is considered to comprise all the calcium that reaches the intestinal tract from within the body and tissues. If it is assumed that the TDJ Ca mixes with the food calcium and that absorption takes place from this mixture, then the following equation is valid:

$$\frac{\text{TDJ Ca}}{\text{Ca intake} + \text{TDJ Ca}} = \frac{\text{average specific activity of faeces}}{\text{average specific activity of plasma}} \tag{11}$$

Thus, the TDJ Ca and also the amount of TDJ Ca that is reabsorbed in the gastro-intestinal tract can be calculated. It will be noted that the endogenous faecal calcium is that part of the TDJ Ca that is actually excreted, that is, not reabsorbed.

It should also be noted that the TDJ Ca can readily be calculated from the endogenous faecal loss and the net digestibility. The equation is as follows:

$$\text{TDJ Ca} = \frac{\text{endogenous faecal calcium}}{1 - \dfrac{\text{net digestibility}}{100}} \tag{12}$$

The assumption that the same proportions of food calcium and digestive juice calcium are absorbed probably is in error, although the extent of this error is not yet known. Studies have shown that plasma Ca^{45} is excreted into all regions of the gastro-intestinal tract; it would seem logical that the calcium excreted into the lower parts of the tract would not have the same opportunity for absorption as does the dietary calcium. Thus, the TDJ Ca as estimated by this method may be higher than the true value.

The situation in regard to digestive juice phosphorus is even more complicated because of excretion into the gastro-intestinal tract of organic forms that may have varying degrees of absorption. ZAMYCHKINA & GRODSENSKY (1955), in studies with dogs, showed that the total phosphorus concentration in bile was 40 to 100 times that in the other digestive juices. At one day after administration of labelled inorganic phosphate to the dog, up to 99% of the radiophosphorus of the bile was incorporated into organic phosphorus compounds, primarily the phosphoslipid, lecithin, which had been synthesized in the liver. Experiments showed that the lecithin of the bile was absorbed into the blood without the phosphorus being split off, but at a slower rate than the absorption of dietary inorganic phosphorus.

C. *Faecal Endogenous Calcium in Cattle*

Table 2 presents some values for faecal endogenous calcium and net digestibility in cattle of various ages as compiled from HANSARD, COMAR & PLUMLEE (1954). The faecal endogenous calcium ranged from about 0·4 g per day in young calves to about 9 g in aged animals. When these values are expressed in terms of milligrams of calcium per kilogram body-weight, the narrow range over the age spectrum indicates a high correlation with body-weight.

Table 2—Faecal Endogenous Calcium and Digestibility of Calcium in Cattle as Function of Age

Age	Calcium, g/day		Faecal endogenous Ca		Digestibility	
mo.	Intake	Faeces	g/day	mg/kg body wt.	Apparent (%)	Net (%)
0·3	8·8	0·6	0·41	12	93	98
1	7·4	1·2	0·97	14	84	98
6	21	16	3·3	18	24	41
15–24	22	19	4·3	14	14	34
25–34	27	22	5·4	17	17	36
35–73	22	22	8·0	18	0	34
144–190	19	24	9·2	21	−28	22

(From HANSARD, COMAR & PLUMLEE, 1954.)

In the animals 6 months of age and over on a normal calcium intake, it is of interest to note that the faecal endogenous calcium represented 20 to 35% of the total faecal calcium. In the younger animals there was apparently less unabsorbed calcium passing through the tract so that the faecal endogenous calcium was a higher proportion of the total faecal calcium. This was probably because the 10-day and 1-month calves were on a milk diet, as will be discussed later. The digestibility values are self-explanatory, indicating an underestimate of the feed value when apparent digestibilities from the balance trials are used. It is interesting to note that the aged animals, although absorbing 22 per cent of the dietary calcium, could not make good the daily loss of 9·2 g of calcium.

There has been interest in the effects of dietary levels of calcium and phosphorus on the faecal endogenous losses. Experiments have shown the following:

(a) When cattle were placed on a very low calcium and phosphorus ration for 2 weeks, the faecal endogenous calcium was not changed but the faecal endogenous phosphorus was markedly reduced.

(b) Cattle on a low-normal calcium intake for 6 months, as compared with animals on a high level, showed no differences in faecal endogenous calcium. After 15 months the animals on the low level had adapted by about a 30% reduction in faecal endogenous loss.

It seems, therefore, that the faecal endogenous calcium is not affected by short-term changes in dietary intake, but over long time periods the lowered endogenous calcium loss may represent an adaptation for the control of calcium economy. On the other hand, faecal endogenous phosphorus losses do reflect the current intake levels.

A practical application of these procedures is indicated in a study of calcium availability as a function of type of diet in calves (LENGEMANN & COMAR, 1954). With calves from 2 to 7 months of age, it was shown that the net digestibility of calcium in a milk ration averaged 84%, whereas the corresponding value for a hay and grain ration was 27%. The results showed clearly that the animals were able to absorb and retain more calcium from milk than from hay and grain. It was also demonstrated that this change in absorption was reversible and occurred rapidly following the dietary change; for example, a 2-month-old calf weaned to hay and grain had a net digestibility of 38%, but 2 weeks after being put back on a milk diet had a value of 92%. There was an indication of similar but less marked results with aged animals.

The faecal endogenous calcium losses may give a rough idea of the degree of renewal of the skeleton. In cattle, the urinary loss of calcium is small and can be neglected for this consideration. Also, it has been shown that the dietary calcium that is absorbed and re-excreted probably does not amount to more than 15% of the endogenous calcium. It then appears from column 5 of Table 2 that about 0·1% of the skeletal calcium is lost daily from cattle (this is calculated from the assumption that 1 kg body-weight contains about 15 g Ca). This value does not have specific physiological meaning because undoubtedly various regions of the skeleton have different rates of renewal. Later, in connection with the long-term removal of strontium from sheep, this matter will be mentioned again.

D. *Faecal Endogenous Calcium in Man*(4)

Data on faecal endogenous loss of calcium in man are available from the work of BELLIN & LASZLO (1953), BLAU *et al.* (1954) and LASZLO *et al.* (1955a).

A 3-months metabolic study was conducted on two normal subjects. A standard low calcium diet containing approximately 150 mg calcium/day was given throughout the entire study. The calcium intake was then raised by the addition of calcium gluconate approximately 5-fold in the second month, and 12-fold in the third month of the study. At the start of the experiment a tracer dose of Ca^{45} was administered orally to both patients. Data obtained by metabolic balances and by the radiocalcium technique are summarized in Table 3.

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(4) This discussion is based upon the paper of D. LASZLO and co-workers entitled, "Biological Studies on Calcium, Strontium, Lanthanum and Yttrium", presented at the Geneva Conference.

The calcium balances were slightly negative in both patients as is usually observed on a low calcium intake. With the increase in calcium intake the balances became progressively more positive and were + 610 mg and + 874 mg/day at the highest intake level for the two patients, respectively. The urinary calcium excretion of the two subjects was in the normal range. Although the urinary calcium increased only slightly at higher calcium intake levels, the calcium content of the stools increased significantly and progressively. Whether this high calcium content of the stools was due to food calcium that passed unabsorbed through the digestive tract, or due to larger amounts of calcium excreted with the digestive juices into the intestinal tract, was clarified by the radiocalcium technique.

Table 3—Metabolism of Calcium in Two Normal Subjects

Calcium mg/day	Patient B			Patient L		
	1st month	2nd month	3rd month	1st month	2nd month	3rd month
Intake	107	529	1446	135	638	1740
Urine	90	85	103	51	66	92
Faeces	151	408	733	162	278	774
Balance	−134	+36	+610	−78	+294	+874
Endogenous faecal	91	91	73	118	67	93
TDJ	160	158	160	365	273	237
Net digest. (%)	44	41	54	67	69	61

(From LASZLO, BLAU, SPENCER & SWERNOV, 1955a.)

The daily endogenous faecal calcium values for the two subjects were: 91 and 118 mg on low calcium intake, 91 and 67 mg on medium high, and 73 and 93 mg on high calcium intake. Therefore, the increase of dietary calcium did not significantly change the endogenous faecal calcium, so that the high faecal calcium was due to unabsorbed food calcium. For Patient B, when the calcium intake was 107 mg/day, the absorbed calcium was 47 mg/day or 44% of the intake; on a medium high intake, 41 per cent; and on the high intake level, 54%. The corresponding values for Patient L were 67, 69, and 61% respectively. This confirms the findings with cattle that the faecal endogenous loss of calcium is independent of short-term dietary changes.

If amounts of calcium are offered that are in excess of the requirements, the body has several mechanisms to deal with this excess load; it can either reduce intestinal absorption, increase renal calcium excretion, or increase calcium excretion by way of the digestive juices. Since neither the urinary calcium nor the endogenous faecal calcium increased significantly in the two subjects when the calcium intake was raised over 10-fold, it may be assumed that the calcium homeostasis is maintained in part through a mechanism that regulates the rate of calcium absorption from the intestinal tract. Information is available that in certain physiologic and pathologic conditions, such as growth, ageing and malnutrition, the utilization of dietary intake may vary according to the requirements of the body. For instance, in osteoporosis of the aged, the utilization seems to be considerably lower than in young adults.

It is of interest to note that on the medium and high calcium intakes, the faecal endogenous calcium was about 23 and 11% of the total faecal calcium. This appears somewhat lower than the corresponding values for cattle.

E. *Movement of Calcium in Developing Chick Embryo*

The source of calcium for the developing chick embryo has been of considerable interest. Detailed studies of the movement of calcium among the various components of the hen's egg during embryonic development have been reported by JOHNSTON & COMAR (1955). The following discussion of this work illustrates the non-mathematical interpretation of a multi-component system in terms of known physiological and morphological concepts.

In general, the procedure has been to inject Ca^{45} into the dense albumen of the fertile egg. At about 24-hour intervals during the hatching period, eggs were sacrificed and samples of yolk, albumen, shell, blood, extraembryonic fluid, and total embryo were analysed for Ca^{45} and total calcium.

The specific activity curves are presented in Fig. 2. The albumen (⊡-⊡) in this experiment represents the original source of labelled calcium and therefore had the highest specific activity (SA) at all times. Experience showed that the Ca^{45} was uniformly distributed in the albumen, certainly by a few days after injection. The definite decrease in SA of the albumen that occurred after six days must have resulted from a loss of calcium from this component with a replacement by calcium of a lower SA from some other component.

It is next of interest to consider the SA of the shell (▲-▲), which showed a peak at about nine days and a rapid decline thereafter. It is probable that the Ca^{45} ions entered the shell by exchange. It must be remembered that the values for the shell in Fig. 2 represent the SA of the total shell and that the SA of the localized inner surfaces was probably much higher. It is also probable that this inner surface would be selectively removed at the time when the shell begins to supply calcium. Thus, the SA of the calcium initially furnished by the shell would be much higher than indicated by the values in Fig. 2. In terms of total activity, the shell accumulated approximately 11% of the dose by the eighth day.

The SA of the total embryo (⊙-⊙) showed a steady rise until the eleventh day and then declined until hatching. In general this means that before the eleventh day, a larger proportion of the calcium ions of the embryo had originated in the albumen pool than after the eleventh day. The SA of the blood (●-●) followed the same general pattern as the total embryo except that it was higher than the SA of the total embryo between 5 and 14 days and slightly lower thereafter. Up to the eleventh day the blood SA curve was very similar to that of the albumen; this curve is consistent with the water movement that occurs during the first 9 to 10 days of development. During the sixth to ninth day the albumen loses a considerable amount of water to the embryo and extraembryonic membranes. The SA of the fluid portion of the blood would necessarily reflect that of the albumen because of the mechanism of blood formation. After the 11-day peak, the blood SA fell rapidly. This probably results from the intense osteogenesis accompanied by a massive utilization of calcium during this period; in addition, since the total amount of calcium in the blood is low at 13 days, the blood SA is very sensitive to any replacement of calcium that occurs.

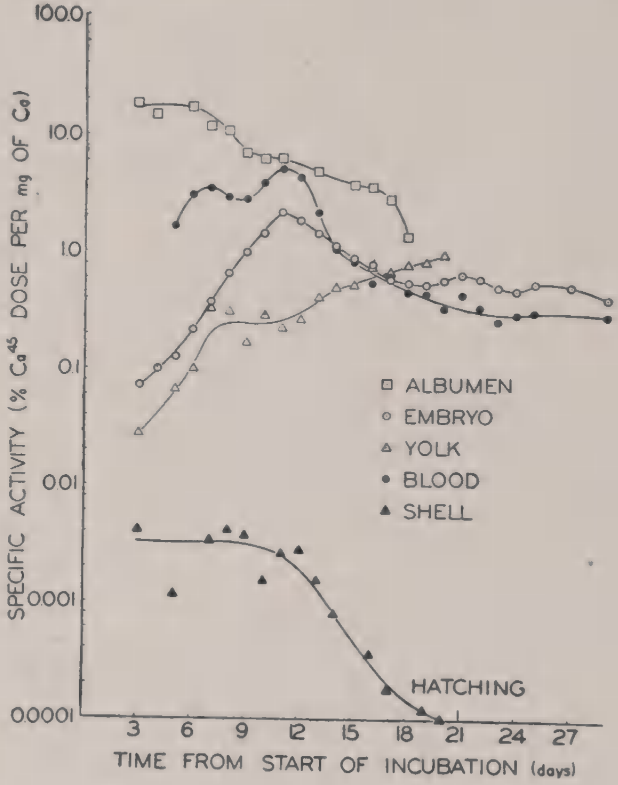

Fig. 2. Specific activities of embryo and egg components during incubation. Calcium 45 was injected into the albumen at start of incubation. (From Johnston and Comar, 1955.)

The SA curve of the yolk (△-△) was characterized by a plateau between the seventh and twelfth days; thereafter it gradually rose until the twentieth day at which time the yolk is drawn through the umbilicus into the body of the chick. The increase in yolk SA during early incubation was probably a result of diffusion of calcium ions from the albumen to the yolk since the curve closely resembles that for water movement from the albumen to the yolk. The yolk is separated from the albumen only by the vitelline membrane during this time. Later the yolk sac and the extended area vasculosa intervene, and additional barriers to diffusion are established. It would appear that the yolk is thereby cut off directly from its former source of Ca^{45}, and any further rise in the SA of the yolk would most likely result from the absorption of labelled calcium from the blood. This situation seems to be indicated by the rise in the SA of the yolk observed between the eleventh and fifteenth days. During this time it appears that the blood is giving up some of its calcium to the reserve pool in the yolk for future use. The continued rise of the SA of the yolk after 15 days may in part be due to the imbibition of extraembryonic fluids by the embryo and to the possibility that some of these fluids find their way to the yolk sac via the vitelline duct. The SA of the extraembryonic fluid was relatively high, about 1·4 at 19 days. This high SA of the extraembryonic fluid probably can be accounted for by the fact that there is an apparent mingling of the contents of the albumen sac, and the amnion and chorioallantois during the last week of incubation.

In summary, the calculations indicate that 85 to 95% of the embryonic calcium accumulated over the first ten days was derived from the yolk. After ten days the shell started to contribute calcium to the embryo. The movement of calcium among various egg components was correlated with accepted morphological and physiological concepts.

These findings are in general agreement with those of Sasaki (1955) who reports as follows: the calcium utilized for the development of embryo in the early stage of growth was mainly derived from yolk and a little from white. Calcium in amniotic and allantoic fluids during the middle stage of incubation was mostly derived from yolk. During the latter half of incubation, more than half of the total amount of calcium collected by the embryo was from the shell, about one-quarter from yolk and the rest from the white and other sources. During the later stage, yolk was a main source of calcium supply to the embryo, while yolk itself was receiving a considerable amount of calcium from the shell. The amount of calcium flowing into the yolk during incubation was approximately the amount contained in the yolk at egg laying.

4. STRONTIUM METABOLISM

The metabolism of the radioisotopes of strontium has been widely investigated because these elements are perhaps the most hazardous of the fission products. This is because radiostrontium is usually well absorbed by the biological system, is deposited primarily in the bones by the growth and/or exchange processes, is transmitted in relatively large amounts to milk and to the developing foetus, and is known to cause bone tumours. There has also been a secondary interest in the use of stable strontium to promote calcium absorption under certain conditions.

Since strontium and calcium have similar and interrelated biological behaviour, comparative data will be presented whenever possible.

A. *Continuous Ingestion of Strontium*

It is important to distinguish between assimilation of strontium over short or over long periods of time. In the latter case the bones will presumably become completely labelled and exchange reactions will play no part. Comar, Whitney & Lengemann (1955) described a study designed to answer a specific practical question: what is the comparative deposition of Sr^{90} and dietary calcium in the body of the newborn and in the skeleton of the growing rat following the continuous ingestion of a normal diet containing carrier-free Sr^{90}? Corollary information was obtained on the contribution of maternal calcium and maternal Sr^{90} to the developing foetus.

In this experiment, two generations of rats were grown on the normal diet with a constant Sr^{90}—Ca ratio at all times. The comparative utilization of the dietary calcium and Sr^{90} has been expressed in terms of a "preferential utilization factor" described below:

$$\text{preferential utilization factor} = \frac{Sr^{90}/\text{Ca in diet}}{Sr^{90}/\text{Ca in bones}} \tag{13}$$

From this study, it was shown that dietary calcium was utilized by rats for bone growth by a factor of 3·6 over Sr^{90}. These results were confirmed by ALEXANDER, NUSBAUM & MACDONALD (1955) who obtained a value of 3·4 by analysis of stable strontium and calcium in the feed and bones of rats. Their values for other species were: mice, 2·9; guinea pigs, 4·5; jack rabbits, 5·0; cotton-tail rabbits, 4·6; kangaroo rats, 6·2. (Note the later discussion of excretion of normal dietary strontium by man.) More rigorous terminology is to be proposed by this laboratory that will allow expression of the discrimination caused by the individual physiological processes, in addition to the overall result.

The continuous feeding experiments with rats on the Sr^{90} labelled feed also indicated that dietary calcium was utilized for bone formation in the developing foetus by a factor of 5·1 over Sr^{90}. As part of this study it was also shown that, under the given conditions, about 30% of the foetal calcium was derived from the maternal calcium, and 70% from the dietary calcium.

B. *Excretion of Radioactive Calcium and Strontium by Cattle*

In contradistinction to the data just presented on long-term comparative utilization of dietary strontium and calcium in the young animal, consideration is now given to the comparative fate of single oral doses of Ca* and Sr*.

Table 4—Faecal and Urinary Excretion of Ingested Ca^{45} and Sr^{89} in Cattle (Double Tracer Experiments)

Age (mo)	Faeces*		Urine*	
	% Ca^{45}	% Sr^{89}	% Ca^{45}	% Sr^{89}
2	23	31	0·072	0·14
3	42	52	0·13	1·1
5	85	87	0·20	0·90
10	83	96	0·42	1·1
144–190	92	100	1·1	2·0

* Values as per cent of ingested dose.
(From COMAR *et al.*, 1954.)

Table 4 shows the faecal and urinary excretion of ingested Ca* and Sr* as a function of age of cattle (COMAR *et al.*, 1954). These results were obtained from double tracer experiments using Ca^{45} and Sr^{89} simultaneously in animals on a normal hay and concentrate ration. It is noted that the faecal excretions of Ca* and Sr* were about the same, although the Sr* values did appear to be consistently higher. The urinary excretions of Sr* exceeded those of Ca* by a factor of 2- to 9-fold. In another study, the faecal excretion (7-day) of twenty yearling steers given Sr^{90} as the chloride orally was found to be 69·9 ± 8·6%;(5) six similar animals excreted 70 ± 7% of an oral $Ca^{45}Cl_2$ dose. The urinary excretion was about 0·2% for the Ca^{45} and about 2·1% for the Sr^{90}.

Table 5—Effect of Diet on Faecal and Urinary Excretion of Ingested Ca^{45} and Sr^{89} by Calves (Double Tracer Experiments)

Age	Diet	Faeces* % Ca^{45}	Faeces* % Sr^{89}	Urine* % Ca^{45}	Urine* % Sr^{89}
2 day	milk	0·2	0·2	0·64	4·9
2 day	milk	0·8	0·4	0·97	6·3
2 mo.	milk	6	14	0·066	0·19
2 mo.	grain	23	31	0·072	0·14
3 mo.	milk	3	9	0·77	3·9
3 mo.	grain	42	52	0·13	1·1
5 mo.	milk	21	36	0·13	0·08
5 mo.	grain	85	87	0·20	0·90
Aged	Low Ca	0	0	0·12	0·76
Aged	High Ca	25	0	0·35	1·2

* Values as per cent of ingested dose.
(From Comar *et al.*, 1954.)

Table 6—Faecal and Urinary Excretion of Intravenously Injected Ca^{45} and Sr^{90} in Mature Dairy Cows Four Days after Administration

	% of Dose in Faeces		% of Dose in Urine	
	Ca^{45}	Sr^{90}	Ca^{45}	Sr^{90}
	17	23	0·46	24
	18	8	1·2	24
	16	—	1·5	—
	21	—	1·0	—
	—	—	—	—
mean	18	16	1·0	24

Note: Ca^{45} values represent 4 trials on 2 animals (Visek *et al.*, 1953b.)
Sr^{90} values are from 2 different animals.

It has previously been pointed out that calves on a milk diet were better able to utilize the dietary calcium. Table 5 shows this effect in terms of excreted Ca^{45}, and indicates that strontium absorption is also increased by the milk diet. The milk ration decreased Ca^{45} faecal excretion from 4- to 14-fold, and Sr^{89} from 2- to 6-fold. The pattern of increased urinary excretion of Sr* is again confirmed in the additional animals reported in Table 5.

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(5) Mean ± mean deviation.

A comparison of the excretion of intravenously injected Ca* and Sr* is available from various experiments with mature dairy cows (Table 6). Because different animals studied at different times are involved, the comparisons are not as good as those previously discussed. However, the general picture seems clear. The excretion of the two radioisotopes into the gastro-intestinal tract was about the same; the urinary excretion of strontium was about 24 times that of the calcium.

C. *Excretion of Radioactive Calcium and Strontium by Man*

It is, of course, not possible to obtain needed information from direct observations on man as has been previously reported for animals. However, some data are available from procedures that are feasible but which may limit the interpretations. Typical studies are as follows: (a) use of low levels of Ca^{45} in mentally deficient patients (BRONNER *et al*., 1954, 1956); this presents some difficulties in radioassay of samples; (b) use of Ca^{45} and Sr^{85} (6) in terminal

Table 7—Excretion of Ingested and Intravenously Injected Strontium by Man

	Per cent of Sr dose		Time	Age	Method	Condition of subject	Ref.*
	Faeces	Urine					
Oral	64	21	30d.	40	Stable Sr; 250 mg	Normal	(1)
	58	18	30d.	50	Stable Sr; 250 mg	Normal	(1)
	68	20	30d.	29	Stable Sr; 100 mg	Normal	(1)
	83	8·8	12d.	54	Sr 85; single dose	Lymphoma, osteoporosis	(2)
	79	15	12d.	60	Sr 85; single dose	Lung Ca.	(2)
	82	3	12d.	59	Sr 85; single dose	Paget's disease of bone	(2)
	60	8·8	—	46	Sr 85; daily dose	Ovarian tumour	(3)
	60	3·2	—	28	Sr 85; daily dose	Leukemia	(3)
	86	3·0	10d.	58	Sr 85; single dose	Ca. breast, osteometastases	(3)
Intravenous	6	65	30d.	40	Stable Sr; 100 mg	Normal	(1)
	0	67	30d.	45	Stable Sr; 20 mg	Normal	(1)
	13	35	12d.	54	Sr 85; single dose	Lymphoma, osteoporosis	(2)
	10	57	12d.	60	Sr 85; single dose	Lung Ca.	(2)
	7	14	12d.	59	Sr 85; single dose	Paget's disease of bone	(2)

* References for this table:—

(1) HARRISON, RAYMOND, & TRETHEWAY (1955).
(2) LASZLO & SPENCER (1955).
(3) COMAR & LOTZ (1955).

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(6) The use of cyclotron produced Sr^{85} offers the advantage over Sr^{89} of a high specific activity preparation without contamination by long-lived Sr^{90}.

patients (LASZLO *et al.*, 1955b; COMAR *et al.*, 1955); the difficulties of extrapolating such results to normal individuals are well recognized; (c) administration to normal subjects of stable strontium in amounts which permit its course to be followed by radioactivation analysis (HARRISON *et al.*, 1955); mass effects must be considered, but the ability to analyse the normal strontium content of diet, excreta, and tissues offers important advantages.

Results on the fate of ingested and intravenously injected strontium are compiled in Table 7. The ages of the subjects were predominantly over 40 years. It may first be noted that after oral administration the faecal excretion ranged from 58 to 86% of the dose, whereas the urinary excretion ranged from 3 to 21%. There were no clear-cut differences between results from stable strontium, single doses of Sr^{85}, or daily doses of Sr^{85}.

After intravenous injection, relatively small amounts appeared in the faeces (0 to 13%) and larger amounts in the urine (14 to 67%). There was a suggestion of higher urinary excretion when the stable strontium method was used.

Table 8—Excretion of Ingested and Intravenously Injected Ca^{45} by Man

	Per cent of Ca 45 dose		Time	Age	Condition of subject	Ref.*
	Faeces	Urine				
Oral	63	7·0	26d.	—	Normal	(1)
	42	4·1	26d.	—	Normal	(1)
Intravenous	7·7	0·9	10d.	68	Ca. prostrate	(2)
	10	2·6	10d.	75	Ca. prostrate	(2)
	12	12	10d.	73	Multiple myeloma	(2)
	13	9·4	10d.	55	Multiple myeloma	(2)
	9·7	10	10d.	63	Ca. lung	(2)
	12	25	10d.	78	Ca. prostrate, osteoporosis	(2)
	15	26	60d.	21	Normal	(3)
	2·7	5·1	5d.	12·6	(Mean values for several boys)	(3)

* References for this table:—

(1) BLAU *et al.* (1954).
(2) LASZLO & SPENCER (1955).
(3) BRONNER *et al.* (1956).

Similar data for Ca^{45} are presented in Table 8. There are too many variables to permit conclusive comparisons between Ca^{45} and Sr^{90}; however, it does seem that the urinary excretion of injected Sr^{90} was higher than that of Ca^{45}. HARRISON *et al.* (1955), from data on the urinary excretion of injected stable strontium, concluded that the renal clearance of strontium is at least three times that of calcium in humans.

HARRISON *et al.* (1955) have also presented some interesting information on the comparative intake and excretion of the normal dietary strontium and calcium. For example, a subject receiving 1·99 mg of strontium per day in the diet excreted about 80% in the faeces and about 20% in the urine; of the 1·18 g of calcium in the diet, there was about 48% excreted in the faeces and 39% in the urine. Since the faecal excretion represents primarily unabsorbed calcium or strontium, it appears that absorption from the gut was almost twice as great for calcium as for strontium.

In general, the following picture emerges from both the human and animal studies. Calcium and strontium are metabolized similarly and are affected by the same variables, such as age, diet, hormones, etc.; but there are differences which operate to favour the retention of calcium in the body. These differences are: (a) preferential utilization of calcium in bone formation—this selectivity may result in part from the following processes; (b) preferential excretion of strontium in the urine, presumably on account of low tubular reabsorption; (c) preferential absorption of calcium from the gastro-intestinal tract; and (d) preferential secretion of calcium into milk.

D. *Pattern of Radioactive Calcium and Strontium Deposition in Bones of Cattle*

In general, the gross deposition in bone of radioisotopes of strontium, calcium, and phosphate is quite similar (COMAR, LOTZ & BOYD, 1952).

The typical short-term distribution of Ca^{45} in the long bone of a young animal is shown in Fig. 3 (COMAR, 1955). The autoradiogram is of a longitudinal section of the metatarsal bone of a 30-day-old calf that had been given orally about 0·5 mc Ca^{45} and sacrificed 7 days later. It is noted that the extent and nature of the radiocalcium deposition is characteristic, and is a reflection of the particular bone structure, as follows: A, heavy and relatively diffuse deposition occurs below the epiphyseal plate, probably as a result of vascularity, increased blood-flow and bone growth in this region. B, the subperiosteal accumulation is characterized by a sharp line of deposition which is related to the narrow layer of growth cells and the limited blood circulation that occurs in this region. C, the area of endochondral bone growth shows a linear deposition (the "funnel"), which, however, is spotty and thereby readily distinguished from the periosteal lay-down. D, the region of trabecular bone shows a more generalized spotty distribution at a lower intensity than the

Table 9—Concentration of Sr^{90} in Tissues of Steers at Varying Times after Ingestion (Values given as per cent of dose per gram fresh weight corrected to a 500 lb body-weight, and multiplied by 10^4)

Tissue	3 days	7 days	1 mo.	4 mo.	12 mo.	18 mo.
Blood	0·065	0·024	—	—	—	0·0002
Liver	0·040	0·012	—	—	—	0·0006
Kidney	0·10	0·029	—	—	—	0·0008
Muscle	0·17	—	—	—	—	0·0005
Femur shaft	3·2	3·0	4·6	5·5	1·7	2·3
Femur epiphysis	5·1	3·4	3·9	4·9	2·3	1·2
Femur head	4·0	4·1	3·7	5·2	1·7	1·5
Mandible	6·9	6·7	8·2	7·4	1·4	0·69
Molar	1·6	1·9	1·5	1·3	1·7	3·6
Ilium	3·4	2·8	3·0	5·5	2·3	2·0
Rib epiphysis	10·1	8·8	7·4	1·5	0·37	0·13
Rib shaft	4·4	4·2	6·1	7·3	1·8	1·0
Vertebra	5·7	4·3	5·3	4·3	0·86	0·54

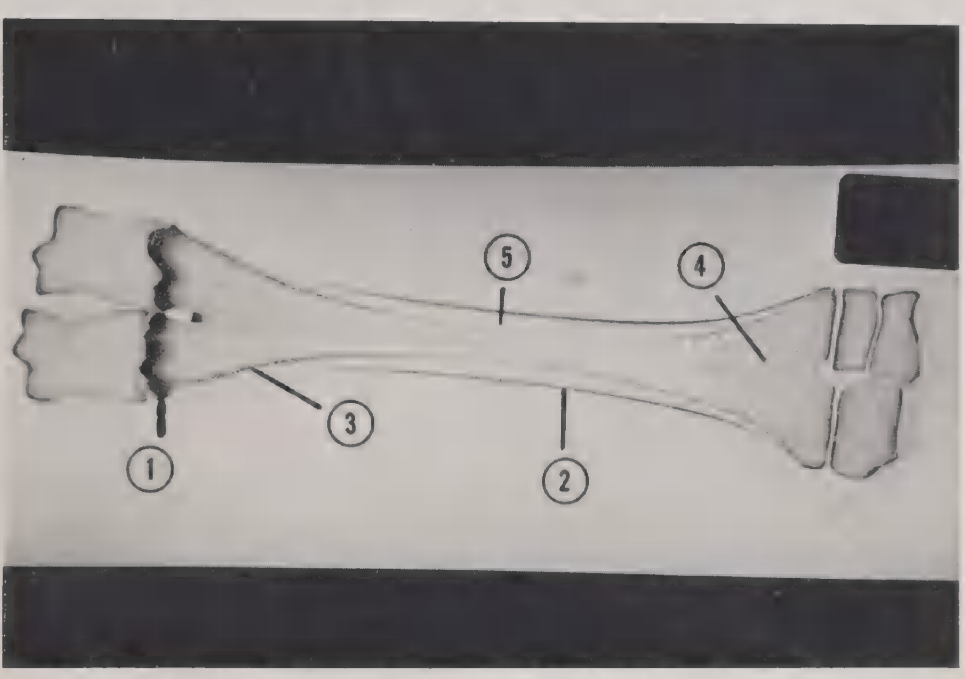

Fig. 3. Ca^{45} autoradiogram of metatarsus of 1-month-old calf sacrificed 7 days after administration. 1, heavy deposition below epiphyseal plate; 2, sharp subperiosteal deposition; 3, spotty deposition in area of endochondral bone growth; 4, trabecular bone; 5, compact bone of shaft. (From COMAR, 1955.)

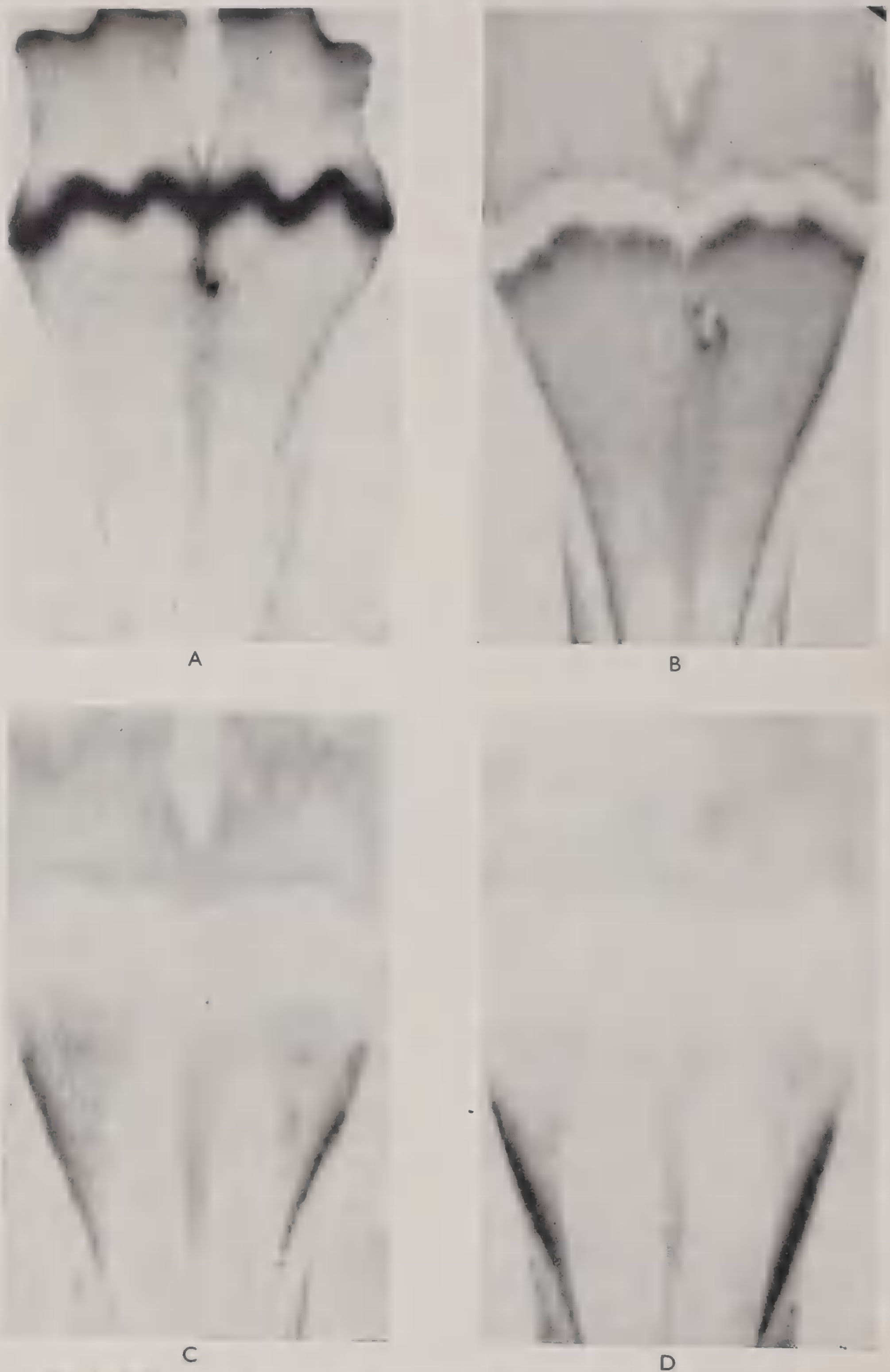

Fig. 4. Sr^{90} autoradiograms of metatarsals of yearling steers. A, 3 days after administration; B, at 4 months note new bone growth; C, at 18 months note retention in "funnel"; D, at 42 months note retention in "funnel", subperiosteal regions and Haversian canals. (From COMAR *et al.*, 1952.)

Plate V

areas already mentioned. E, the compact bone of the shaft shows little radioactivity, probably as a result of poor circulation; however, there is activity in this region.

To study the long-term retention of Sr^{90}, yearling steers were given 5 or 10 mc of this radioisotope orally and sacrificed at varying times thereafter (Comar *et al.*, 1952). Table 9 presents the results of radioassay of tissue samples. The important finding is that the concentration in bone decreased on the average only by a factor of about 3 over the 18-month period. The soft tissues, of course, showed very low concentrations after a few days.

The pattern of removal is demonstrated in Fig. 4, which presents autoradiograms of the distal end of metatarsals from animals in this experiment. Thus, visual confirmation is provided for the bone values, which show a marked loss with time from the epiphyseal regions where resorption occurs and a smaller loss from areas of compact bone. The degree of darkening is not a measure of comparative concentrations because of differences in photographic processing. The significant observations are: (a) the apparent retention of the radioactivity in the region where originally deposited, (b) the low concentration of Sr^{90} in the new bone mineral, (c) the removal by resorption of the Sr^{90} originally deposited in the primary spongiosa, and (d) the retention, even after 42 months, of radioactivity deposited in the "funnel", the subperiosteal region and the Haversian canals.

E. *Long-term Excretion of Radiostrontium*(7)

If individuals should be exposed to radioactive strontium, it would be helpful to be able to estimate their initial body burden from subsequent excretion curves. A mathematical analysis has therefore been made of excretion data from a yearling sheep having received a known dosage (1 mc Sr^{90}) intravenously. Fig. 5 presents the total excretion on a semi-log plot expressed as microcuries per day, as a function of time up to about 550 days. After the first few days, the faecal and urinary excretion rates were about equal.

This curve was resolved in the usual way into 5 components. For each rate process, it was possible to determine the half-time, the excretion rate at zero time, and the total amount that was destined to be excreted at this rate process. The general equations for each rate process are as follows:

$$U = U_0 e \exp\left[-(0{\cdot}693/T)t\right] \tag{14}$$

where U = excretion rate (μc/24 hours) by the given rate process at time t.

U_0 = same as above at zero time.

T = half time (days) of the given rate process.

It then follows that

$$Q = \int_0^\infty U_0 e \exp\left[-(0{\cdot}693t/T)dt\right] \tag{15}$$

$$= \frac{(U_0)(T)}{0{\cdot}693} \tag{16}$$

where Q = radioactivity (μc) that is to be excreted by the rate process.

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(7) The mathematical analyses in this section were done by P. E. Brown of the Health Physics Division of the Oak Ridge National Laboratory.

The values as calculated from the curve and equations are presented in Table 10. For purposes of over-all accuracy, the excretion values for the first day were excluded from the mathematical analysis. It is noted that when the first-day values are included in the summation of Q values, there is good agreement with the known dose; similar agreement was obtained for the summation when the entire curve was used. Thus the excretion data in this case did give a good estimate of the initial body burden. The half-times of the five rate processes ranged from 0·5 to 1 200 days.

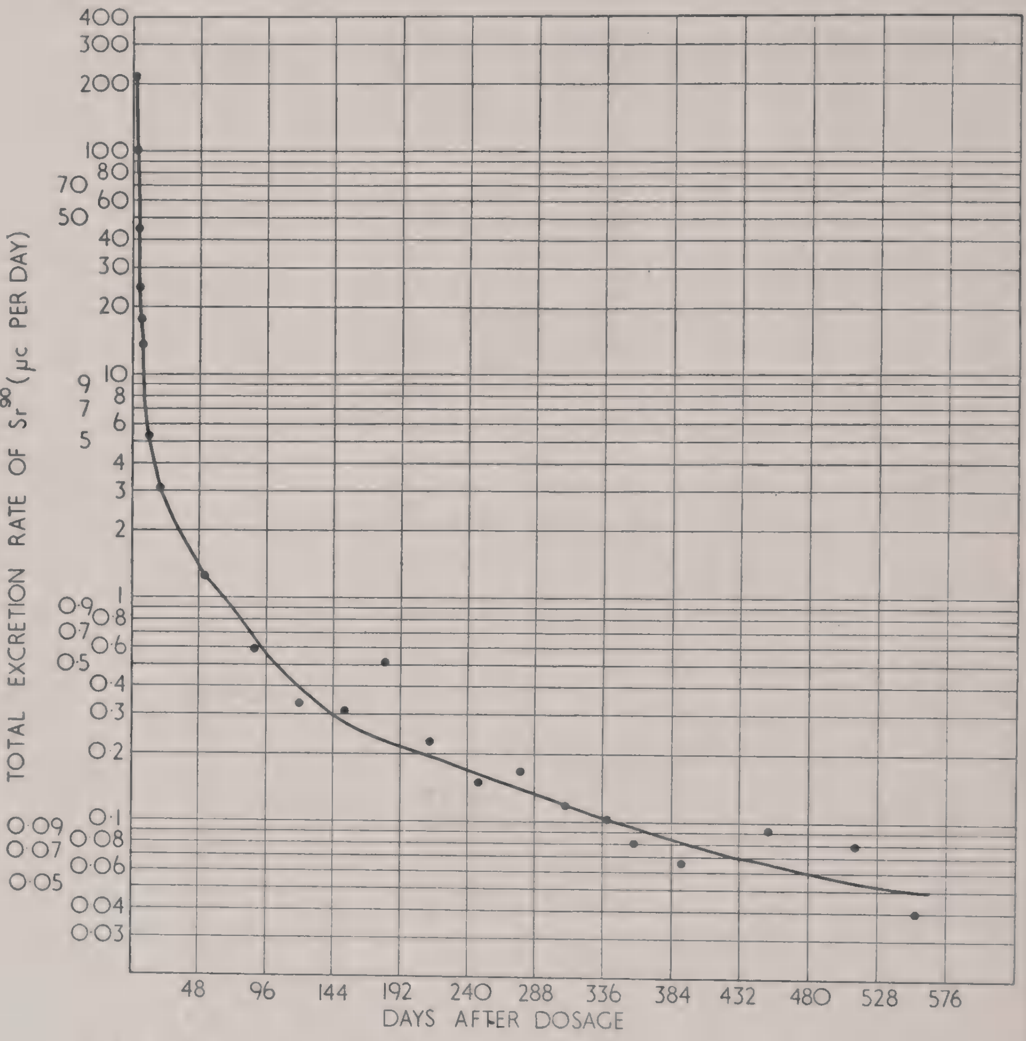

Fig. 5. Total excretion rate of intravenously injected strontium 90 by sheep.

It is of interest to note that a high proportion (about 45%) of the excretion takes place soon after exposure. This means that early excretion data should be available if this method is to be practicable. The uncertainties of interpretation of this type of analysis are well recognized. It is pointed out that even after 550 days the slope of the curve will probably change, adding still further components to the curve which, however, would not be expected to introduce any considerable change in the values reported in Table 10. The data also give a straight line on a log-log plot and can thus be represented by a power function, as was shown by NORRIS, SPECKMAN & GUSTAFSON (1955) for long-term radium excretion. The report of LOONEY *et al.* (1955) is an excellent review of radium and thorium excretion data.

Table 10—*Analysis of Excretion of* Sr^{90} *by Sheep*
(1 000 μc *injected intravenously*).

Rate Process	T	U_0	Q
	(Half-time in days)	(Excretion at O time in μc/day)	(Amount excreted in μc)
1	0·50	350	253
2	1·5	74	158
3	6·8	12·5	84
4	59	1·2	102
5	1200	0·117	203
			800
		Amount excreted first day	197
		Total	997

In summary, it appears that a mathematical resolution into exponential functions can be used to estimate the initial body burden and to give some idea as to rates of excretion as a function of time. More information is needed for correlation between physiological function and excretion rates, although the concepts previously discussed in regard to skeletal metabolism may explain the excretion rates, at least non-quantitatively. It is of interest to note that between 11 and 20 days the animal excreted about 1% of its body burden per day, whereas at about 400 days the value was about 0·04%. The calcium turnover from faecal endogenous values would be estimated at about 0·1% of body calcium per day. Thus, the deposited radiostrontium was at first more easily removable than the average bone calcium, and later was less easily removable.

F. *Secretion of Radioactive Calcium and Strontium into Milk*

This discussion is limited primarily to information regarding the appearance of ingested radiocalcium and radiostrontium in the milk of the dairy cow, and observations on the effect of dietary calcium level on radiostrontium secretion. While such studies are of prime importance for an understanding of the physiology of milk secretion, emphasis is placed on quantitative aspects to enable evaluation of health hazards when dairy animals are exposed to known intakes of radiostrontium.

The fate of Ca^{45} after administration to the lactating goat and dairy cow has been reported in detail by VISEK, BARNES & LOOSLI (1952) and by VISEK, MONROE, SWANSON & COMAR (1953b), respectively. When Ca^{45} was injected into dairy cows at about 20 days prepartum, much of the radioisotope was deposited in the mammary gland where it remained until the colostrum and milk were collected. This observation is of particular interest in showing the existence of calcium pools in which the calcium ions are not interchangeable with the circulating calcium ions.

In general, it was shown that the amount of blood Ca^{45} deposited in the milk was directly correlated with the total amount of milk calcium secreted For example, during 4 days after intravenous injection, a cow producing 4 litres of milk per day secreted 19% of the Ca^{45} dose, whereas when the same cow and another one produced about 7 litres per day, 34% was secreted Therefore, all results are expressed as per cent of ingested dose per litre of milk, since this tends to compensate for differences in milk production when data are to be compared.

Fig. 6 shows the appearance of Sr^{90} in the milk secreted during 30 days after ingestion of a single dose. The shape of the curve, with a peak at 2 days, is quite similar to that reported for Ca^{45}.

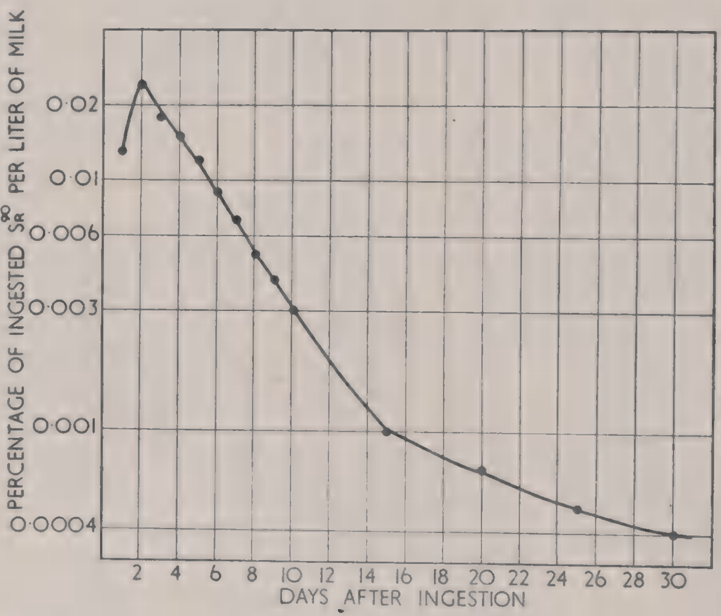

Fig. 6. Secretion of ingested strontium 90 in the milk of the dairy cow. (From Comar *et al.*, 1954.)

Although most experimental studies have charted the course of a single dose of radioactivity, there is a practical interest in what happens when the animal receives a daily intake of radiostrontium. Theoretically, if the cow receives the same dose of radioactivity each day, then the amount secreted in each day's milk should reach a plateau value; this value should be equal to the summation of the recoveries on successive days following a single dosage. The time to reach the plateau following daily intake is essentially the time required for the amount of a single dose appearing in the milk to fall to a small value. In the case of Sr*, 7 days has been chosen as a convenient time. To calculate the percentage of the daily intake of Ca* or Sr* found per litre of milk, the total radioactivity in the milk collected during 7 days after dosage is divided by the average number of litres produced per day. By these calculations, the single dose data can be used to predict the behaviour of the daily dose. Experimental data with P^{32} in hens (O'NEIL *et al.*, 1948) and daily dose studies reported in Table 11 confirm this method of calculation.

Table 11 summarizes the amounts of Ca^{45} and Sr* secreted into the milk on the basis of per cent of a daily dose per litre of milk. Under normal dietary conditions, with a daily calcium intake of about 20 g, it was found that approximately 1·4% of a daily ingested dose of Ca^{45} was secreted per litre of milk.

Table 11—*Comparison of Secretion of Orally Administered Ca*^{45} *and Sr* in the Milk of the Dairy Cow*

Animal No.	Daily Ca intake (g)	Daily milk, litres	Per cent of Daily intake per litre of Milk		Method
			Ca^{45}	Sr*	
1	Approx. 17	4·7	1·2	—	Single dose
1	,, 17	4·3	1·4	—	Single dose
5	,, 17	5·6	1·6	—	Single dose
5	,, 17	7·0	1·3	—	Single dose
4	,, 17	—	1·5	—	Daily dose
			Avg. 1·4		
10	25	7·9	—	0·26	Daily dose
5	25	16	—	0·18	Daily dose
—	—	—	—	0·16	Single dose
				Avg. 0·20	
10	106	7·9	—	0·14	Daily dose
10	138	7·9	—	0·10	Daily dose
10	100	7·9	—	0·16	Daily dose
5	100	16	—	0·12	Daily dose
				Avg. 0·13	

(From COMAR *et al.*, 1954.)

Under similar conditions, only about 0·2% of the daily ingested Sr* was found per litre of milk; this value of 0·2% may be useful in estimating the amount of radiostrontium to be found in the milk when the intake in the feed is known. Thus, there was a preferential secretion of ingested Ca^{45} over Sr^{90} by a factor of 7. Although the comparative absorption from the gut and urinary excretion appear to favour calcium, as already mentioned, it does not seem that these effects are large enough to account for the differential milk secretion; this would suggest a selective physiological mechanism, probably in the actual secretion process.

An important practical aspect is concerned with the possible use of increased dietary calcium to reduce the amount of radioactive strontium entering the milk. Accordingly, a study was undertaken in which the daily calcium intake was increased from 25 to about 100 grammes. The experiments with animals No. 10 and 5, as reported in Table 11, were done at about the same time under similar conditions so that the results should be comparable as a test for the effect of calcium level. It is noted that there did appear to be less strontium in the milk at the higher level of calcium. However, the effect was small and certainly not indicative of a simple dilution phenomenon. An increase of calcium from 25 to 100 grammes per day only caused a decrease of about 35% in the secretion of the labelled strontium. This same effect has been observed by MacDonald *et al.* (1954) in studies of effect of mass of calcium on deposition of Sr^{90} in the skeleton of the rat.

G. *Deposition of Radioactive Calcium and Strontium in the Hen's Egg*

The pattern of radiocalcium deposition in the hen's egg was reported by Comar & Driggers (1949). An over-all comparison of Ca^{45} and Sr^{90} deposition after ingestion by the laying hen is given in Table 12; these data were from different animals.

Table 12—*Deposition of Ingested Radiocalcium and Radiostrontium in the Hen's Egg*

Days between ingestion and laying of egg	Percentage of dose					
	Shell		Yolk		White	
	Ca^{45}	Sr^{90}	Ca^{45}	Sr^{90}	Ca^{45}	Sr^{90}
1	30	28	0·002	0·05	0·1	0·01
3	1	2·5	0·14	0·13	0·006	0·008
4	1	1·5	0·17	0·15	0·003	0·005
5	1	1·0	0·11	0·09	0·002	0·007
8	0·5	0·5	0·02	0·04	0·002	0·002

(From Comar *et al.*, 1954.)

It is clear that the two radioisotopes were handled similarly with the major accumulation in the shell of the egg laid after ingestion. Additional data will be required to determine whether the apparent preferential deposition of Ca^{45} in the white of the first day's egg is reproducible.

5. IODINE METABOLISM AND THYROID FUNCTION

The dominant role of iodine in thyroid physiology and the importance of the thyroid gland in animal metabolism has been recognized for many years. The isolation of thyroxine in 1919 aroused tremendous enthusiasm in the study of the biochemistry of thyroid hormones; this enthusiasm had waned considerably by 1938 primarily because of technical difficulties in isolating and characterizing the substances involved. The availability of radioiodine, in conjunction with such methods as paper chromatography and paper electrophoresis, has again stimulated interest in studies of the nature, formation and metabolism of thyroid hormones. Discussion is limited here to some of the newer concepts and problems which have been developed primarily through use of radioisotopes. In addition the practical problems of measurement of thyroid activity in livestock and secretion of radioiodine into milk are considered. Space will not permit more than an indication of the individual studies which led to the ideas presented, nor will it be possible to treat the whole area of thyroid economy and hormonal interrelationships. The following reviews may be consulted for details and more complete coverage: ROCHE & MICHEL (1955), LEBLOND (1948), and EDELMANN, CURTIS & KOSHLAND (1954).

A. *Biosynthesis of Thyroid Hormones*

Within recent years an important discovery has been that another hormonal substance besides thyroxine (*l*-3, 5, 3′, 5′-tetraiodothyronine) is present in the thyroglobulin of the thyroid gland; namely, *l*-3, 5, 3′-triiodothyronine. Quantitatively, depending upon the test, this newly found substance is from three to six times more active than *l*-thyroxine. Even more recently, two other substances have been found in the rat thyroid: *l*-3, 3′-diiodothyronine which has about the same activity as thyroxine using the goitre prevention assay, and *l*-3, 3′, 5′-triiodothyronine which has one-tenth of the thyroxine activity.

The biosynthetic process can be considered stepwise, as follows: entry and concentration of iodide by the thyroid gland; assimilation of iodine within the gland; and formation of the iodinated amino acids and hormones. First must be considered the process by means of which the gland can concentrate iodide from plasma. The nature of this process is unknown but is possibly similar in principle to the accumulation of ions against a concentration gradient which other organs accomplish.

It has been suggested that either enzyme activity or binding by a specific protein may be responsible. Efficient concentration occurs only in intact cells. The process is reversible as indicated by the ability of thiocyanate to cause release of iodide into blood. The concentration of iodide in the gland is considered as an individual step since, for example, propylthiouracil-treated animals can concentrate iodide but cannot subsequently convert the iodide to the thyroid hormones. Thiocyanate, on the other hand, prevents concentration of iodide. Iodide ions appear to penetrate into the bordering, or acinar cells, of the thyroid gland prior to rapid incorporation into organic compounds.

The concentrated iodide ions in the gland must be oxidized to a reactive form before becoming organically bound; this reactive form is probably elemental iodine. An oxidizing enzyme is most likely the responsible agent although no specific enzyme has been characterized. As an analogy, it has been found that the addition of iodine to unboiled fresh milk results in the iodination of the casein, due primarily to the action of xanthine oxidase. Such iodination does not occur in boiled milk.

The reactive iodine reacts immediately with thyroglobulin at the site of formation to produce iodinated amino acids, including *l*-thyroxine and *l*-3, 5, 3′-triiodothyronine. The steps of this reaction have been established by studying the time pattern of formation of different iodinated constituents following radioiodine injection, a task virtually impossible without the use of radioisotopes. After injection of I^{131} into the rat, the formation of labelled *l*-3-monoiodotyrosine reaches a maximum at about 6 hours; the formation of labelled *l*-3, 5-diiodotyrosine is slower with a pattern suggesting that monoiodotyrosine is the precursor. The *l*-thyroxine and *l*-3, 5, 3′-triiodothyronine are detectable quite later in a manner suggesting that the iodotyrosines are the iodothyronine precursors. Also, it appears that, at least in thyroglobulin, *l*-thyroxine is not the precursor of *l*-3, 5, 3′-triiodothyronine; if anything, the opposite is indicated. The details of the sequence are not yet clear, particularly in regard to enzymatic mechanisms and the nature of substrates involved. There is little question but that the circulating *l*-thyroxine is of thyroid origin. However, there is considerable question as to the origin of plasma *l*-3, 5, 3′-triiodothyronine. As a constituent of thyroglobulin, triiodothyronine is first liberated by proteolysis and then secreted by the thyroid. However, since the triiodothyronine diffuses freely from the body fluids to the cells, there has been the suggestion that part of the circulating triiodothyronine might arise from partial deiodination of *l*-thyroxine in receptor cells.

B. *Metabolism of Thyroid Hormones*

There is no thyroglobulin in blood under normal conditions. Proteolysis of thyroglobulin, presumably by an enzyme (thyroid cathepsin) liberates the hormones for secretion into the circulation. The thyroid hormones contain at least 90% of the total plasma iodine; about 75% of the organic plasma iodine is *l*-thyroxine, with *l*-3, 5, 3′-triiodothyronine being the other main constituent.

Thyroxine secreted by the gland is predominantly bound by gamma-globulin, but may possibly be combined with several gamma-globulins. This was shown in electrophoretic patterns of plasma from patients who had received I^{131}.

In contrast to *l*-thyroxine, *l*-3, 5, 3′-triiodothyronine is not combined selectively with any protein. Of particular importance is that triiodothyronine diffuses much more rapidly from plasma into the tissue cells. Because of this rapid diffusion, CHAIKOFF, TAUROG & WHEAT (1955) found it necessary to sample thyroid venous blood in order to obtain detectable levels of labelled triiodothyronine after intravenous radioiodine injections into large animals. Thus it was possible to sample the plasma draining the thyroid before dilution had occurred with the entire circulatory volume. Immediately following the removal of the thyroid venous blood, carotid arterial blood samples and the thyroid gland were taken. Sensitive techniques, such as filter paper chromatography and autoradiography, were necessary to separate and measure the small quantities of labelled substances in these specimens. The relative amounts of triiodothyronine and thyroxine in thyroid venous blood directly reflected their proportions in the thyroid gland. Triiodothyronine iodine usually was about 10% of the thyroxine iodine in the venous blood, while in the thyroid gland this value was quite similar, being about 14%. Triiodothyronine was never detected with certainty in carotid arterial blood. In this study, it was observed that the thyroxine I^{131} concentration was twice as great in the thyroid vein as in the carotid artery. Conversely, the concentration of iodine I^{131} was less in the vein than the artery, indicating active removal of iodine by the gland.

The iodotyrosines that are liberated from the gland are found only in trace amounts in the plasma; this observation was also confirmed by CHAIKOFF *et al.* (1955). They are probably degraded in the thyroid due to the presence of deiodinase, an enzyme that removes iodide from the iodotyrosines. The liberated iodide ions are essentially reutilized for biosynthesis of the hormones since almost all of the iodine leaving the gland is in hormonal form.

Degradation of the thyroid hormones systemically yields their iodine content to the blood as iodide. Part of it will be collected again by the thyroid for entrance into the hormonal cycle; most of it, however, will be excreted by the kidneys.

The liver plays an important role in the metabolism of the thyroid hormones. This organ has the ability to destroy excess hormones with the liberation of iodide and, in addition, to remove thyroxine and triiodothyronine from plasma through a regulatory mechanism of the enterohepatic circulation. Certain phases of their biliary excretion pattern have been characterized through the use of labelled radioisomers and paper chromatographic techniques. Thyroxine and triiodothyronine labelled with I^{131} were found in the bile either unchanged or as the phenolic glycuroconjugates or as degradation products. The phenolic glycuroconjugates can be hydrolysed by spleen β-glycuronidase to the free hormone and the glycuronic moiety. The degradation products were identified as the iodothyropyruvic acids which probably resulted from the oxidative deamination of the parent compounds by *l*-amino oxidase or by a transamination reaction. Kidney and other organs are responsible for some destruction of thyroid hormones.

C. *Estimation of Thyroid Activity in Livestock*

The interrelationship between thyroid function and lactation in the dairy cow has been well emphasized. Also, the relatively recent interest in the use of thyroid inhibitors in the production of more obese livestock has prompted the development of methods for estimation of thyroid activity in large animals. Such a procedure for the estimation of thyroid secretion rate in intact sheep has been described by Reineke & Henneman (1955). The method is based on the inhibition of thyroidal I^{131} output by progressively increasing doses of exogenous thyroxine. The sheep is injected with I^{131}, and 7 to 10 days later the thyroid gland is counted externally. On 3 to 4 successive days, increasing amounts of thyroxine are given and the gland counted each day. The thyroxine secretion rate was estimated by computing the thyroxine dosage required to maintain the thyroid count at 100% of the initial level.

In general, ewes had a higher thyroid secretion rate than did males. The rate was increased significantly during lactation, and was higher in 2-year-old than in 4-year-old ewes. The thyroid secretion rate declined 4- to 5-fold from the winter to the summer months. In growing ewe lambs, a significant positive correlation was found between thyroid secretion level and growth rate.

Blincoe & Brody (1955) have discussed several methods for estimation of thyroid activity in cattle. The four parameters used are listed as follows: (a) rate of I^{131} uptake by thyroid, (b) rate of release of I^{131} hormone from thyroid to blood, (c) maximum uptake of I^{131} by the thyroid and (d) ratio of thyroxine-like I^{131} to total I^{131} in plasma. The last method was considered to be the best. All of these methods avoid the administration of thyroxine, which the authors felt might itself affect the metabolic rate under their conditions.

In the previously mentioned study by Chaikoff *et al.* (1955), it was observed that, in one group of sheep and a dairy calf, I^{131} thyroxine was not present in any appreciable amount in the thyroid vein 24 hours after injection of I^{131}. This slow release of I^{131} thyroxine was accompanied by a fairly rapid accumulation of I^{131} by the thyroid. The question has been raised as to whether thyroidal I^{131} uptake alone is a good index of thyroid function. This agrees well with the opinion of Blincoe & Brody (1955) that the ratio of plasma thyroxine-like I^{131} to the plasma I^{131} may be better index than some of the other cited methods.

D. *Secretion of Radioiodine into Milk*

Small amounts of radioactive iodine from atomic bomb debris have been measured in the thyroid glands of livestock (Van Middlesworth, 1954); also, the grazing animal might be exposed to fission-product iodine accidentally emitted from atomic energy plants. It is therefore of practical interest to estimate what proportion of I^{131} ingested by the dairy cow will appear in the milk and thereby reach the consumer. This discussion is concerned primarily with the secretion of ingested I^{131} into the milk of the dairy cow under normal conditions, and some of the factors which may affect the amounts entering the milk. The data were obtained from Lengemann, Monroe & Swanson (1955a, b).

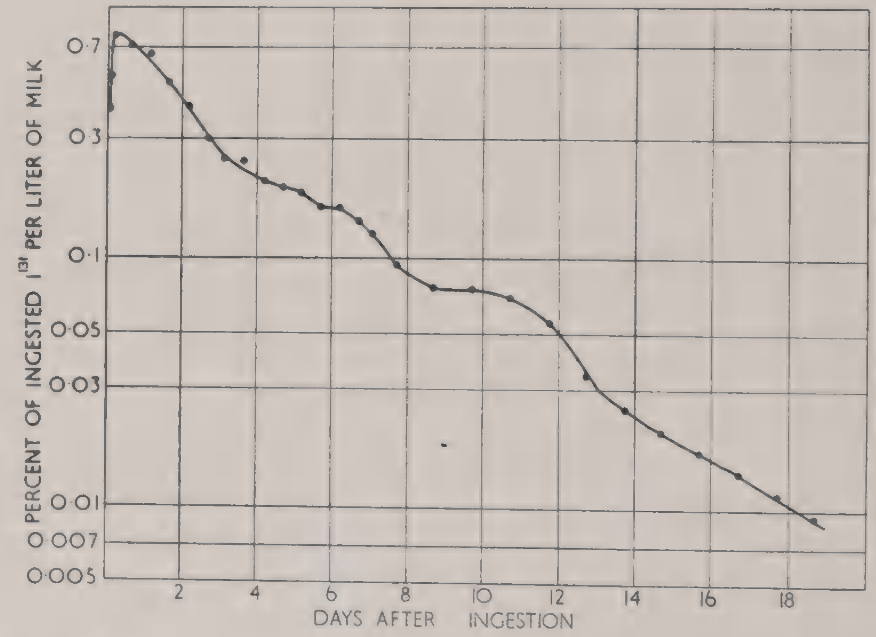

Fig. 7. Secretion of ingested iodine 131 in the milk of the dairy cow. (From Lengemann, Monroe & Swanson, 1955b.)

1. *Pattern of secretion*—A typical curve for the appearance of ingested I^{131} in the milk is presented in Fig. 7. Attention is first called to the early appearance of the isotope in the milk (30 minutes) and the short time after dosage (3 to 4 hours) at which the peak concentration occurs. This behaviour is

almost identical with that noted for radiofluorine (Perkinson *et al.*, 1955), in which case it was demonstrated that considerable absorption occurred from the rumen.

The portion of the curve showing the decrease in radioiodine appearing in the milk indicated a half-period of roughly 1·5 days for the first 3 to 4 days, and after that a half-period of about 3 days. This is in general agreement with the findings of Glascock (1954a) who suggested that the slower release at the later times may be accounted for by release of iodine in chemical combination from the thyroid gland. Time disappearance curves of radioactivity in the blood non-protein fraction, milk, urine and faeces were generally parallel. The level at 7 days after dosage was sufficiently reduced so that the summation of daily collection values over this time period was used for calculation and comparison of data, as discussed for radiostrontium.

2. *Oral vs. intravenous administration*—Four cows were given radioiodine intravenously and orally with sufficient time between trials to avoid interference from residual radioactivity. In the 7-day collection period, the amount of I^{131} in the milk after oral dosage averaged 85 ± 5% $^{(5)}$ of that after intravenous administration.

The difference was found to be in the milk secreted during the first few hours after dosage. After 16 hours, the percentage of I^{131} in the milk was very much the same regardless of the method of administration. The governing factors in this comparison then appear to be the high blood concentration of the radioisotope shortly after intravenous injection, and the high degree of absorption of iodine from the gastro-intestinal tract.

3. *Fate of radioiodine in the dairy cow*—Table 13 presents an over-all summary of the behaviour of radioiodine after administration to the lactating dairy cow.

Table 13—Over-all Summary of Fate of I^{131} in the Dairy Cow

	No. of cows	No. of trials	Per cent of dose at 7 days*	Average production, litres per day
Milk	11	32	6·2 ± 2·0	7·9
Thyroid	11	23	15 ± 6·0	—
Urine	7	17	55 ± 14	—
Faeces	7	17	17 ± 7	—

* Mean ± mean deviation.

(From Lengemann, Monroe & Swanson, 1955b.)

These averages cover observations on 7 to 11 animals in 17 to 32 separate trials, as indicated. The animals were on routine management and dairy rations; in about half of the trials the cows were confined to metabolism stalls described by Comar (1955). Results are included for different stages of lactation, different levels of milk production, different seasons of the year, and both oral and intravenous administration. However, the values for per cent of dose in milk were all corrected to the oral basis by use of the 85% factor previously described. Thyroid measurements were done by external scintillation counting.

It is noted that about 6% of the dose was found in the week's milk following ingestion. This average value is subject to seasonal variation, as will be shown in the next section. Calculating on the basis of daily ingestion, one would expect to find about 0·8% of the daily dose per litre of milk at the plateau. The values of 15% for accumulation in the thyroid, and 55 and 17% for excretion in the urine and faeces, respectively, are not unexpected from experience with other animals. However, WRIGHT, CHRISTIAN & ANDREWS (1955) reported that, in a single experiment, a goat eliminated 45·3%, 40·7%, and 2·3% of ingested I^{131} in the milk, urine and faeces, respectively.

4. *Factors affecting radioiodine secretion in milk*—In general, the stage of lactation and the amount of milk produced had no clear-cut effect on the amount of radioiodine secreted into the milk. In contrast, it will be remembered that radiocalcium and presumably radiostrontium secretion was directly related to milk yield. Thus there is not the advantage in expressing I^{131} results on a per litre of milk basis as was done for calcium and strontium.

The over-all iodine metabolism is apparently sensitive to seasonal changes. Table 14 is arranged to show such effects on the secretion of radioiodine into milk. The Spring–Summer values were in each case higher and averaged about 1·4 times the winter values. The summer period of increased I^{131} in milk is generally considered a period of decreased uptake in the thyroid gland. It is not felt that the decreased summer milk production is a direct factor in this comparison; however, the possibility cannot be ruled out.

Table 14—*Effect of Season on Secretion of Radioiodine into Milk*

Cow No.	Daily milk yield litres		Per cent of dose in 7 day milk	
	Winter	Spring–Summer	Winter	Spring–Summer
20	10	7·3	4·3	5·5
435	10	4·5	6·4	11·4
454	11	8·7	4·1	4·8
23–4	8·2	6·8	5·7	7·4
19	10	5·5	4·7	5·9
Mean			5·0	7·0
Standard error of the mean			±0·4	±1·2

(From LENGEMANN, MONROE & SWANSON, 1955b.)

The effect of large amounts of dietary iodide upon radioiodine secretion into milk was studied with two cows by daily administration of the isotope. The basal ration supplied about 15 mg of iodine per day. At 6 days after the start of I^{131} dosage, the amount of I^{131} had practically reached a plateau as theoretically expected; at 10 days cow A and cow B were given 2 and 4 g per day, respectively, of iodine as sodium iodide in addition to the usual dose of isotope. The iodine supplementation was continued for several days, and the results are summarized in Table 15. It is clear that the massive amounts of extra iodide caused relatively small decreases (about 30 and 50% reduction) in the amount of I^{131} entering the milk. Other evidence indicated that no differences were observable when comparing animals receiving 2 and 20 mg of iodide per day.

5. *Determination of iodine content of tissues by isotope dilution*—A modification of isotope dilution, based on repeated dosage of isotope to give steady state conditions, has often been employed as a substitute for difficult chemical analysis (COMAR, 1955; VISEK *et al.*, 1953b). LENGEMANN & SWANSON (1955) have applied this method for estimation of the iodine content of milk, the thyroid gland and various blood fractions of the dairy cow.

Radioiodine of known specific activity was administered to the animals daily for 7 to 12 days. At this time it was considered that steady state conditions had been attained and the specific activities in all samples were equal. The iodine content of each sample was determined simply by measuring the I^{131} in the sample and dividing by the known specific activity.

In two cows, it was found that milk and whole blood contained an average of 17·4 and 15·1 μg iodine per 100 ml, respectively. The thyroid gland contained about 7·1 μg iodine per mg tissue. When the same cows were given high levels of iodine (2 and 4 g), the total iodine in the milk and whole blood were 2 000 and 2 990 μg per 100 ml, respectively. These results indicate that ingested iodide, even in large amounts, is well absorbed and significant quantities are transferred to milk. This explains why stable iodine is relatively ineffective in reducing the amounts of ingested radioiodine that enter the milk.

Table 15—Effect of Massive Amounts of Stable Iodide on Secretion of Radioiodine into Milk as Shown by Daily Dosage Studies

	Per cent of daily ingested dose	
	Total milk	Per litre of milk
Cow A		
Normal diet	4·7	0·74
+ 2g NaI	3·3	0·54
Cow B		
Normal diet	9·4	1·3
+ 4g NaI	4·5	0·62

(From LENGEMANN, MONROE & SWANSON, 1955b.)

6. *Form of iodine in milk*—GLASCOCK (1954a) reported that the I^{131} secreted in the milk of the dairy cow was in the form of the iodide ion as shown by failure of the radioactivity to separate with the protein or fat fraction. LENGEMANN *et al.* (1955a) reported that the radioiodine in milk was removable by an ion-exchange resin. WRIGHT *et al.* (1955) found iodide 131 ion in the milk of goats treated with I^{131} labelled iodocasein or sodium iodide, but was unable to detect radioactivity as monoiodotyrosine, diiodotyrosine, or thyroxine.

F. *Some Physiological and Biochemical Properties of Astatine*^{211} (8)

Astatine^{211} is an alpha-particle-emitting 7·3 hour isotope, produced by the bombardment of bismuth by 29-MeV alpha particles (Bi^{209} (d, 2n) At^{211}). Because of the juxtaposition of astatine and iodine in the periodic table, extensive investigations reported by HAMILTON, DURBIN, ASLING & JOHNSTON (1955) have dealt with the possible interrelationship between astatine and iodine in thyroid physiology. It was shown soon after the availability of At^{211} that the thyroid of rats, guinea pigs, monkeys, and man can selectively accumulate this element, but to a lesser degree than I^{131}. Factors that influence I^{131} metabolism are similarly effective on At^{211}, such as pituitary thyrotrophin, low iodine diets, exogenous thyroxine, and thiocyanate. An exception is the enhancement of At^{211} retention in the thyroid by the thiouracil-type goitrogens rather than a lowering as in the case of iodine. Recently, it has been found that, in the normal thyroid, about two-thirds of At^{211} is rather firmly bound or "stable", and about one-third is quite labile. The "stable" At^{211} is associated with the thyroglobulin fraction, suggesting that it is bound in some manner to this protein. The labile fraction may be elemental astatine adsorbed on the cellular protein, or possibly may be the astatide ion.

The degree of radiodestruction of the thyroid gland by At^{211} was also studied by HAMILTON *et al.* (loc. cit.). The amount of damage was estimated with two criteria: (a) by measuring the ability of the remaining thyroid tissue to accumulate a tracer dose of I^{131} and (b) by weighing and histologically examining the tissue. Six days after the injection of varying levels of At^{211} (0·4 to 1·8 μc/g body-weight), it was observed that iodine uptake diminished as the dose of At^{211} increased. This reflected the functional damage to the gland. The maximal effect of thyroidal I^{131} uptake was reached at the dose level of 1·2 μc/g body-weight which is close to the MLD 60. Over longer periods of time, however, lower doses were equally effective in impairing thyroid function. This suggests that the earliest phenomena of irradiation damage by At^{211} are related to impaired cellular metabolism while the subsequent processes reflect cell death and glandular atrophy. This was borne out by the observation that, early after At^{211} administration, the weights of the thyroid glands were reduced only slightly from control values; yet, at one year post-injection, there was a marked reduction in thyroid weight. The early histological picture of the lower dose groups was of a TSH-stimulated gland with the epithelium of the follicles being unusually high, abundant vacuolation of follicles, and dilatation of the capillary vasculature. In the six-day high dose level (1·2–1·8 μc/g body-weight), disintegration and cellular destruction of thyroid tissue was in evidence. In the chronic studies (one year after At^{211} administration), appreciable glandular damage was evident at the lowest dose levels. At the higher dose levels after a year, little tissue was observed that resembled thyroid follicles. Vascularity was poor and most of the tissue fibrous. It was interesting to note that the parathyroids were consistently normal.

(8) This discussion is based upon the paper of J. G. HAMILTON and co-workers entitled "Physiological and Biochemical Studies of Astatine–211 (Eka-Iodine)", presented at the Geneva Conference.

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8

THE USE OF RADIOISOTOPES IN ANIMAL BIOCHEMISTRY

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A NEW era in research has been opened up by the introduction of the use of radioisotopes in biochemistry to label molecules. Early work has already allowed one to acquire many fundamental data, which would doubtless not have been possible by classical methods of chemistry.

Two main fields, those of metabolic reactions and their physiological control, have particularly benefited from an extensive use of labelled molecules. In the first, the possibility of following one or several radioelements, during the course of changes comprising the metabolism of a molecule or at the time of its synthesis, has opened a very important field of action. The origin of the radicals constituting organic substances which are relatively complex, like uric acid and heme of the haemoglobins, has been defined with precision. Ideas as new and as fundamental as the participation of carbon dioxide in some processes of synthesis in animal tissues have been established, thanks to the use of labelled molecules. In the field of physiological chemistry, the introduction into an organism or into autonomous cells, such as erythrocytes, of radioactive molecules in concentrations causing no disturbance to cellular activity, started the study of the mechanism controlling metabolism in satisfactory conditions, formerly often inaccessible. Thus the fate of hormones, circulating in plasma in minute concentrations only, has been determined without the conditions of use by the tissues undergoing modification due to their administration in non-physiological doses.

Without touching on all the subjects concerning the use of radioisotopes by biochemists, the papers presented at the International Conference on the Peaceful Uses of Atomic Energy covered a very wide range. Moreover, a study of the papers allows a consideration at the same time of the possibilities of use of radioactive elements in animal biochemistry. The subjects studied have been collected in three groups in order to make their presentation more clear.

I. USE OF LABELLED MOLECULES IN THE STUDY OF METABOLIC REACTIONS

The investigations devoted to the study of reactions, leading to the synthesis or degradation of molecules independently of the localization in tissue of these reactions, are those in which the advances have up till now been most extensive. At the conference, four communications covering this field were presented.

It had been thought that the proteins of tissues are capable of taking up amino acids without undergoing extensive hydrolysis, and it was even inferred from this possibility that a state of equilibrium might exist between proteins and amino acids of the body fluids. Such a problem could not be completed experimentally except with labelled amino acids, and that was the object of the work of U. N. OREKOVICH and his collaborators (1955). The injection of tyrosine 1–C^{14} into fertile hen-eggs has been followed by the incorporation of this substance into the proteins of the embryo after 13 days incubation, but not into those of the white or yolk of the egg. One does not therefore observe any renewal of the tyrosine in the proteins of these two media. It is the same with glycine and methionine, shown by experiments carried out under the same conditions with the former labelled with C^{14} in the carboxyl position and with the latter containing S^{35}. Conversely, the incorporation of tyrosine into egg proteins has been easily followed during the course of their formation *in vivo* after administration of labelled amino acids to laying hens. A special problem bearing on the formation of procollagen and collagen and modifications under the action of scurvy has been studied by the same author. Deficiency in vitamin C leads to a disturbance of the formation of collagen and its precursor, a disturbance of which a biochemical aspect is manifest as a very marked diminution of the speed and intensity of the inclusion of labelled glycine in proteins.

Biosynthesis of nucleic acids was the subject of a paper presented by G. B. BROWN (1955). This communication summarized many investigations from the point of view of synthesis. The participation of adenine, as the only free purine capable of being incorporated in nucleic acid, was worthy of note, and the use of the ribonucleic fraction was discussed at length. Some reactions of transpurination, effecting the transfer of a purine from one nucleotide to another, have been disclosed with the help of the use of nucleotides labelled in their various constituents (base, pentose, phosphate). They exemplify the remarkable selectivity with which it is possible to study the elaboration of complex molecular associations by a relatively simple experimental technique. The role of the "active metabolite" in the synthesis of nucleic acids appeared to be held not by free purines but by nucleotides, of which those containing adenine are without doubt the most important.

The use of sulphites by higher animals has been studied by P. FROMAGEOT & F. CHAPEVILLE (1955) in conjunction with research on the incorporation of S^{35}, administered as $S^{35}O_4Na_2$ or $S^{35}O_3Na_2$, into cysteinesulfinic acid, taurine and cystine. The first substance has been identified in the aqueous extract of various organs after injection of labelled sulphite but not sulphate. Labelled taurine was absent in the bile in both cases. Cystine isolated from the hydrolysed protein of various organs was demonstrated to be radioactive after injection of sulphite but scarcely or not at all radioactive after sulphate. The sulphur from sulphite is thus only recovered in the amino acid and the comparison of specific activities of cysteinesulfinic acid, cystine and taurine shows that the first is the precursor. Cysteinesulfinic acid could be reduced in cystine. From this it follows that the oxidation of the last is reversible.

An example of the direct observation of the formation of a very labile combination, constituting an intermediary in a reaction of activation of a substrate by an enzyme, is given in the communications of D. E. KOSHLAND (1955). The activation of a carboxyle studied in this respect is of great biological interest because it is essential for the formation of peptides as for the condensation of acetate with the —SH group of co-enzyme A, a process taking part in the synthesis of fatty acids. The source of energy essential to maintain the reactions is hydrolysis of adenosinetriphosphoric acid (ATP); but we wondered for some time how its transport could be effected. Research with the help of carboxylic substances labelled, in their COOH by O^{18}, has enabled this to be determined: it is shown that a glutamyl phosphate is the intermediary indispensable to the formation of the combination of enzyme with glutamic acid and it catalyses the processes of condensation.

Another important aspect of the mechanism of the action of enzymes is the definition of the forms of their reaction with the substrate. In the case of hydrolysis of ATP by myosine, it has been established that mineral phosphate, formed in the presence of H_2O^{18}, contains only O^{18} which is absent from the ADP liberated. This proves that the reaction admits the formation of a combined phosphoryl-enzyme and that the broken bond is that connecting the terminal phosphorus to the oxygen bridge fixed to ADP.

Thus the use of labels in research on metabolic reactions and their mechanisms is shown by the work briefly summarized above. It deals as much with general processes (OREKHOVICH *et al.*, 1955; BROWN, 1955) as with amino acids and their derivatives (FROMAGEOT & CHAPEVILLE, 1955) and with the mode of action of enzymes (KOSHLAND, 1955).

II. THE USE OF LABELLED MOLECULES FOR THE STUDY OF METABOLISM OF TISSUES OR ORGANS

The co-ordination of reactions in the tissues leads to a degree of complication of the metabolic processes such that classical chemistry only allows one to see the end results. The molecules identified by radioelements conserve up to a point their individuality in complex biological media; so the study of tissue metabolism when they are used could have remarkable advantages. Four relevant papers were presented at the conference.

Some recent applications of tritium to biochemical research were demonstrated by GLASCOCK (1955) in four carefully chosen examples. Synthesis of fatty acids in slices of mammary tissues of the rat in the presence of $1-C^{13}-2$ tritioacetate (CH_2T-C^{13} OONa) and of glucose $1-C^{14}$ has been studied as far as it concerns the action of glycerol and insulin on rate processes. Some important differences between the incorporation of C^{14} and CH_2T are recorded in the two cases, and so one can follow the use of each one in the synthesis. The C^{14} of the glucose predominates slightly in the presence of insulin, but CH_2T is much more important in the presence of glycerol, so that the two products exercise their stimulatory action on the synthesis of fatty acids by two independent mechanisms. The metabolism of hexoestrol, a synthetic oestrogen, has been followed after injection of tritiohexoestrol into the rat, and so it has been possible to demonstrate the advantage of its administration compared with stilboestrol, a derivative endowed with similar biological properties. Finally, the transport to milk through the mammary gland of alimentary fatty acids labelled with tritium has been studied in the goat.

The role of adipose tissue has been considered within the framework of metabolism of lipids by B. SHAPIRO & G. ROSE (1955). This tissue uses preformed higher fatty acids so very readily that it cannot synthesise them in the absence of acetate and pyruvate. Furthermore, the formation of glycogen at the expense of glucose is active in adipose tissues, above all after a period of starvation.

Two general papers, one on the metabolism of muscle, the other on that of the brain, were presented by eminent Russian biochemists. A. V. PALADIN & G. E. VLADIMIROV (1955) summarized the most important work carried out in U.S.S.R. by means of isotopes on functional biochemistry of the brain. Experimentation *in vivo* is in this field more rational than that carried out on tissue slices. It has been possible only with the help of P^{32}. Its uptake is much greater in animals whose cortical function is well developed and in the most active central nervous regions. The problem of the chemical topography of the brain, studied for 30 years by A. PALADIN, has similarly been investigated. The study of phosphoprotein of the cortex was in this connexion of special interest. The extension of work to the different stages of ontogenesis and phylogenesis has been successfully completed. Stimulation and inhibition of the functions of the nervous centres have been tied up with the metabolic processes in an important collection of experiments concerning the incorporation of various labelled substances with subjects asleep and awake, with and without electrical stimulation of the centres, anaesthetised, in hibernation, and deficient in vitamin C or E.

The application of radioactive isotopes to the study of the biochemistry of muscle has been achieved by Ferdman and his collaborators, who compared normal tissue and atrophied muscles following denervation or vitamin E deficiency. The speed of renewal of muscular proteins with the exception of collagen has been shown to depend on the functional state of the tissue. The classical idea of the diminution of the continuity in proteins of the muscle ought to be supplemented with another, namely the modification of the dynamic state of muscular proteins in the course of atrophy or dystrophy, since important modifications of metabolism of methionine are demonstrable with S^{35}.

A field previously almost inaccessible to biochemistry has thus been opened by research of which several examples were presented: the dynamic aspect of metabolism at the level of the organs and tissues.

III. USE OF LABELLED MOLECULES IN THE STUDY OF THE METABOLISM OF AN ORGANISM AND GENERAL BIOLOGICAL PROCESSES

The problems posed by the metabolism of the whole organism or by some reactions of the latter have been achieved by the same techniques. Five papers were devoted to these problems. A. BAIRD HASTINGS (1955) presented the general framework of the use of isotopes in biological and medical research with particular emphasis on several fields where their usefulness was greatest. Measurement of the volume of extracellular fluids, of plasma and of circulating red cells, has been achieved, the first by means of injection of salts containing a radioactive atom (Cl^{36} or Na^{24}), the second by marking the plasma proteins with I^{131} or Cr^{51}, or the red cells by Fe^{59} (incorporated in haemoglobin). The biochemistry of bones and teeth has been re-examined by the use of P^{32} or Ca^{45} and the exchanges and incorporation into the bone or dental salts have been followed. In the same way the turnover of plasma proteins, cellular nucleic acids, and lipids has been studied.

The general conclusion is that numerous constituents of organisms, for so long considered "stable and static" are, in effect, in a state of dynamic equilibrium of which the characteristics are peculiar to each tissue and each molecule. The study of this equilibrium leads without any doubt to the understanding of the metabolic co-ordination existing between the various tissues of an organism and to the knowledge of the impact that it could have on a pathological process specifically affecting an organ.

The field of mineral metabolism was the object of much work reviewed by C. L. COMAR (1955), who reviewed certain work devoted to the biochemistry of calcium. The idea of permanent exchange of this between bones and body fluids has been established with the use of labelled calcium, the fate of which has been followed in some organisms. Similarly the mobilisation of endogenous calcium has been demonstrated for the formation of the tricalcium phosphate of milk or for maintaining acidbase equilibrium. Similarly also he follows the calcium metabolism of the embryo during the course of its development. Analogous problems have been investigated by R. SASAKI (1955) to study the fate of P^{32} and Ca^{45} in laying hens. Each of these elements is laid down when the diet is adequate, otherwise bone salts are mobilised. Not only normal processes but also metabolic disturbances can be investigated with radioisotopes as shown by a communication by DE WITT STETTEN (1955). It dealt chiefly with research on gout, creatinuria and diabetes.

The use of radioisotopes in immuno-chemistry in work on antibody synthesis was described by F. J. DIXON (1955). The fate of antigens labelled with I^{131}, C^{14}, S^{35}, and P^{32} was studied, and similarly the synthesis of antibodies in which are incorporated labelled amino acids. In addition, the reactions of antigens and antibodies *in vivo* and *in vitro* have involved, with profit, the labelled molecules.

The papers presented allow one to understand the relevance of radioelements for animal biochemistry and medical applications. Although the results already obtained are numerous and important, a future full of promise remains open to research on the use of labelled molecules.

A new mode of work has been instituted by their introduction in biochemistry and that has been proved by an exceptionally rapid phase of development. For biochemists the use of radioisotopes, already very extensive, will become within a few years as current as that of the microscope became for naturalists in the 19th century.

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